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PRINCIPAL INVESTIGATOR: Tracey F. Weisberg, M.D.

CONTRACTING ORGANIZATION: Maine Medical Center Research Institution
South Portland, Maine 04106

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Tracey F. Weisberg, M.D.

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13. ABSTRACT *(Maximum 200 words)*

The purpose of this research is to determine the role of human growth hormone (hGH) and insulin-like growth factor 1(IGF-1) in the development of an immunodeficient mouse model for human breast cancer. Human GH and IGF1 may be critical to the initiation and progression of tumor growth *in vivo.*

Results suggest that it is questionable whether rhGH alone or in addition to estrogen has a significant role in the development of a primary tumor or the progression of tumor growth in the animal model. In addition, growth hormone may be semi-inhibitory to growth for tumors dependent upon estrogen. Exogenous IGF1 however, enhances the time to development of a palpable primary tumor and likely has a role in sustaining tumor growth and size over and above what has been achievable with estrogen alone. The effect of human rhGH and IGF1 on tumor IGF1, IGF2 and IGFR is currently under evaluation in this laboratory on tumor specimens obtained from the experimental animals. Over the next year in this laboratory, primary tumors from patients under care at Maine Medical Center, will be place into the *scid/scid* mouse model and supplemented with IGF1 to establish if our preliminary results can be applied to the development of new xenograft models.

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Terry F. Wursthg MD 10/14/98

PI - Signature Date

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1.0 INTRODUCTION:

1.1 Subject:

Specific genes within the immune and endocrine systems are likely to be the major controlling elements in the successful development of mouse models for mammary tumor xenografts. We believe that growth factors, specifically human growth hormone (hGH) and Insulin Like Growth Factor (IGF1) may be critically important in the successful establishment of such xenografts in an animal model.

1.2 Purpose:

The purpose of this research is to determine the role of hGH and IGF-1 in the development and maintainence of an immunodeficient mouse model for human breast cancer.

1.3 Scope

Human breast cancer growth in animal models is dependent upon an intact GH/IGF-1 axis. Based upon our preliminary data, we believe that hGH may be critical to the initiation of a primary breast neoplasm *in vivo*. IGF-1 may be critical to maintaining tumor growth *in vivo*. When the GH/IGF-1 axis is interrupted or impaired, tumor growth may become more directly influenced by 17-β estradiol.

To test the hypothesis, the following sets of experiments have been executed to date:

Experiment 1/Specific Aim1: Determine the amount of recombinant human growth hormone (rhGH)that needs to be administered to the experimental animal to result in (1) early engraftment of palpable tumors and (2) accelerated growth of the tumor in the *scid/scid* mouse model, and to correlate serum GH and IGF1 levels with tumor IGF1 and IGFR levels by northern and western analyses. Experiments include administration of rhGH both by continuous infusion and by daily administration to mimic the normal circadian rhythm of human growth hormone.

Experiment 2/Specific Aim 2: To determine the role of IGF1 in the initiation and/or the progression of primary breast cancer growth in a *scid/scid* mouse model and to correlate serum IGF1 with tumor IGF1 and insulin growth factor receptor (IGFR) levels by northern and western analyses.

Experiment 3/ Specific Aim 3: To determine the dose of 17-ß estradiol administration critical to tumor engraftment and progression of growth in *scid/scid* mice that have an impaired GH/IGF1 axis and if exogenous 17-ß estradiol can futher enhance tumor growth in animals administered optimal concentrations IGF1 and/or rhGH.

The following experiments will be executed throughout the second year of funding:

Experiment 4/Specific Aim 4: (to be carried out in the next planned year of research: To grow primary breast cancer explants in the optimized animal model.

1.4 Background

Development of Animal Models For The Study of Human Breast Cancer: Since the original report by Rygaard and Povlsen (2) that congentially athymic nude (*nu/nu*) mice supported the growth of a human colon adenocarcinoma following subcutaneous injection, these T cell-deficient animals have been utilized as experimental hosts for a great variety of human neoplasms. However, there has been only limited success in utilizing *nu/nu* mice as hosts for primary human breast carcinomas. In an extensive study of 262 infiltrating ductal carcinomas, Giovinella et al (3) found that only 6.1% of such primary carcinomas could be grown in *nu/nu* mice following subcutaneous injection. Moreover, the human breast carcinomas that did grow successfully in

nu/nu mice commonly failed to display metastatic properties (4). It has been suggested that the variability in success of metastatic human tumor growth in *nu/nu* mice may be due to background modifying genes (5) that may influence the growth of the human tumors or the metastasis of such tumors. Since there has been only limited success in growing human breast tumors in *nu/nu* mice, preliminary experiments have examined the growth of such tumors in C.B.17 mice homozygous for the severe combined immunodeficiency (*scid*) mutation. C.B17-*scid/scid* mice lack T as well as B cells. Initial data are promising since cell line derived human breast carcinomas show increased take rates and grow faster in *scid/scid* mice than in *nu/nu* mice (6). However, such studies have been limited to the C.B17-*scid/scid* mouse. An added benefit to establishing a breast cancer model in this animal is that the *scid/scid* mouse can have its bone marrow reconstituted with human hematopoietic cells. This feature of the *scid/scid* mouse would allow this animal model to be used in experiments studying the role of human growth factors and cytokines in supporting or impairing human primary tumor growth and the process of metastasis. Use of non-obese diabetic (NOD) *scid/scid* mice may prove to be a superior animal for such experimentation due to impaired natural killer (NK) cell activity in addition to impaired B and T cell function.

The Role of Human Growth Hormone In Human Breast Cancer: A variety of growth factors have been identified that are mitogenic for breast cancer cell lines *in vitro*. The focus of this experimental work is to establish if alterations in the hGH/IGF-1 axis can be made that facilitate the engraftment and subsequent growth of a primary human breast cancer explant in an immunodeficient mouse model. Focus on the hGH/IGF-1 axis in the experimental animals is selected as an area of importance based upon the results of recent experimental results reviewed below. Endocrine glands providing estrogen, progesterone, glucocorticoid, and insulin are prominent regulators of mammary tissue growth. Moreover the protein hormones of the human lactogenic series - pituitary prolactin (PRL) and growth hormone (GH) plus placental lactogen (PL) are of unique importance because of their species specific biological properties (7). GH has been implicated as a growth factor for human breast cancer (8) and it has been shown that rhGH stimulates breast cancer growth through IGF-1 and possibly other growth factors (9). *In vitro*, insulin growth factor receptor (IGF-R), IGF-1, IGF-2 and insulin have all been shown to be mitogens of MCF-7 breast cancer cells (10). The mechanism of this perturbation is unknown, however, it is known that insulin is capable of altering the cell cycle kinetics of MCF-7 human breast cancer cells by facilitating their transit through the G1 phase of the cell cycle (11). *In vitro* it has been shown that estrogen and progesterone may alter the growth of breast cancers by regulating the insulin growth factor binding proteins (IGFBP) and thereby change the carcinoma's responsiveness to IGF-1 (12). In human studies, hGH (8) and IGF-I has been shown to be elevated (13) in operable patients with breast cancer in comparison to uneffected control patients and hGH, IGF-1, IGF-2, and IGF-R levels may be indicators of prognosis or response to treatment (14,15). In another study however, experimental results suggested that in postmenapausal women with breast cancer, the plasma sex steroids fail to influence the concentrations of IGF-1 or IGFBP-1 when present in physiologic concentrations (16). Tamoxifen, an estrogen receptor blocking drug widely used in the adjuvant, metastatic and preventitive management of breast cancer has been shown to have a role in the regulation of the GH axis (17). Tamoxifen decreases serum hGH and IGF-I serum levels in treated patients as well as reduced IGF-I in target organs by a mechanism that is pituitary independent (17). These studies all seem to suggest that GH and insulin growth factor(s) may be critical to the establishment of an optimal millieu for the initiation and promotion of breast neoplasia in a patient.

2.0 BODY OF WORK

2.1 METHODS: Specific Aim 1

Specific Aim 1: Determine the amount of rhGH that needs to be administered to the experimental animal to result in (1) early engraftment of palpable tumors and (2) accelerated growth of the tumor in the *scid/scid* mouse model, and to correlate serum GH and IGF-1 levels with tumor IGF-1 and IGF-R levels by northern and western analyses. Experiments include administration of rhGH both by continuous infusion and by daily administration to mimic the normal circadian rhythm of human growth hormone.

Establishment of MCF7R mouse models

The MCF7R human breast cancer cell line was used in these experiments. MCF7R cells are derived from the parental cell line MCF7. MCF7R cells are rendered resistant to chemotherapeutic drugs due to upregulation of the multiple drug resistant gene 1 (mdr-1 gene) and p-glycoprotein. This cell line was established by gradually forcing MCF7 cells resistant to vincristine. It was a gracious gift from Dr. William Hait, Yale University. The animals models were established by injection of 1 X 10^6 MCF7R cells suspended in Matrigel (Becton Dickinson) into the mammary fat pad of experimental animals 2 days after the initiation of rhGH administration. Animals were assessed weekly for development of tumor growth. Tumors were measured using Vernier caliper. Tumor volumes at each measurement were calculated using the equation

$$v=\pi r^2 l$$

where v is volume, r is the radius of the tumor and l is the length of the tumor.

When tumor growth in experimental and control animals reached 1 X 1 X 1 cm, the animals were euthanized by CO_2 anesthesia, the tumors harvested from the animals, and total RNA extracted.

Selection of experimental animals

Scid/scid mice, *scid lit+/-* mice, *scid/scid lit//lit* and TghGH *scid/s*cid mice were used in this experimental aim. NOD *scid/scid* mice served as true experimental control animals. *Scid/scid lit/lit* animals are animals that have inability to produce gonadatropin hormone releasing hormone and also have ineffective production of growth hormone. TghGH *scid/scid* mice are transgenic mice for human growth hormone. *Scid lit+/-* mice are heterozygotes for the *lit/lit* mutation. All animals were obtained from The Jackson Laboratory, Bar Harbor Maine. Dr. Wesley Beamer has developed colonies of TghGH *scid/scid* mice and *scid/scid lit/lit* mice in his laboratories. Funding from this research effort has made it possible to obtain animals from Dr. Beamer.

Administration of recombinant human growth hormone

Mice were divided into two experimental treatment groups. In the first group, recombinant human growth hormone (rhGH) was administered at the onset of the dark cycle of the room in an attempt to approximate the circadian release of growth hormone in the experimental animals. A second set of animals was treated with continuous infusion of rhGH through Alzet miniosmotic pumps. A dose finding study of rhGH administered to *scid/scid lit/lit* mice established that a 10ug rhGH injection into the peritoneal cavity of *scid/scid lit/lit* mice for three consecutive days resulted in serum human growth hormone levels between 1-2.5ng/ml as measured by the Kallestad Quantitope HGH kit (Sanofi Diagnostics, MN). Due to budgetary restraints in this project, the target dose of rhGH of 5ng/ml was financially impossible to achieve.

Group I animals were injected with a daily dose of rhGH of 1.5ug for two weeks and then every other day for the duration of the experiment (12 weeks). Serum IGF-1 levels were determined with IGF1 By Extraction (Nichols Institute, CA) twice during the 12 week experimental period. Group II animals had Alza pumps (model 1002, 0.25ul/hr, 14days) surgically implanted into the subcutaneous tissue on the posterior thorax of the experimental animals and changed every two weeks throughout the duration of the experimental period. The pumps were loaded with 100ul of rhGH, 0.25ug/ml. Serum IGF-1 levels were determined with *IGF1 By Extraction* (Nichols Institute, CA) twice during the 10 week experimental period.

Northern Analysis for IFG1R

Total RNA as well as mRNA was probed with P^{32} labelled DNA probes specific for IGF1 and IGFR. Probes for IGF1 and IGFR were made from plasmids containing the sequences of interest obtained from ATCC (ATCC, Maryland). Unfortunately, these studies were unsuccessful due to presumably the very low copy number of IGF1 and IGFR. Alternative strategies were developed for their measurement. See next section please.

RT-PCR Assay for IGF1, IGFR and IGF2

For this assay, tumor RNA was extracted using the Tri Reagent (Sigma, St. Louis). Primer sequences for IGF1, IGFR and IGF2 were constructed as per previously published sequences (25). RT-PCR reactions were optimized to produce optimal amplification of the desired targets.

RNA Protection Assay

From our experience with northern analysis, we hypothesized that the sequences that we wished to detect were present in experimental samples in very low copy number. In order to quantify IGF1, IGFR and IGF2 under these conditions, and to also quantitiate changes in their copy number under our experimental conditions, an RNA protection assay is in the process of being developed. Probes for the protection assay are the nested PCR products obtained from above for IGF1, IGFR, and IGF2. The PCR products are cut from the gel and gel purified. Using the sense primer only, the PCR product is reamplified, this time with incorporation of P^{32}.

The RNA protection assay is currently being optimized for all three probes. The specific procedures are well detailed (26).

2.1 METHODS: Specific Aim II

Specific Aim II:To determine determine the role of IGF-1 in the initiation and/or the progression of primary breast cancer growth in a *scid/scid* mouse model and to correlate serum IGF1 with tumor IGF2 and IGFR levels by northern and western analyses.

Establishment of MCF7R mouse models

The MCF7R human breast cancer cell line was used in these experiments. MCF7R cells are derived from the parental cell line MCF7. MCF7R cells are rendered resistant to chemotherapeutic drugs due to upregulation of the multiple drug resistant gene 1 (mdr-1 gene) and p-glycoprotein. This cell line was established by gradually forcing MCF7 cells resistant to vincristine. It was a gracious gift from Dr. William Hait, Yale University. The animals models were established by injection of 1 X 10^6 MCF7R cells suspended in Matrigel (Becton Dickinson) into the mammary fat pad of experimental animals 2 days after the initiation of rhGH administration. Animals were assessed weekly for development of tumor growth. Tumors were measured using Vernier caliper. Tumor volumes at each measurement were calculated using the equation

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where v is volume, r is the radius of the tumor and l is the length of the tumor.

When tumor growth in experimental and control animals reached 1 X 1 X 1 cm, the animals were euthanized by CO_2 anesthesia, the tumors harvested from the animals, and total RNA extracted.

Selection of experimental animals

Scid/scid mice, *scid lit+/-* mice,and *scid/scid lit//lit* mice were used in this experimental aim. NOD *scid/scid* mice served as true experimental control animals. *Scid/scid lit/lit* animals are animals that have inability to produce gonadatropin hormone releasing hormone and also have ineffective production of growth hormone. Because they have decreased production of murine growth hormone, they have ineffective production of murine IGF1 (and likely IGF2). *Scid lit+/-* mice are heterozygotes for the *lit/lit* mutation.

Administration of human IGF-1 to experimental animals

Mice were treated with human IGF-1 (Bachem,CA) by continuous infusion via Alza miniosmotic pumps (Alza pump model number 1002). Prior to beginning the experimentation, a dose finding study of IGF-1 in the *scid/scid lit/lit* was performed. In this experiment it was determined that approximately 2000ng IGF-1 administered daily for three days resulted in a serum

level if IGF1 of 128 ng/ml as measured by *IGF-1 By Extraction Kit* (Nichols Institute Diagnostics ,CA). The anticipated target dose initially planned upon was 200ng/ml. Because of financial restraints a daily delivered dose approximating a serum value of 65 ng/ml was delivered.

Alza pumps (model 1002, 0.25ul/hr, 14days) were surgically implanted into the subcutaneous tissue on the posterior thorax of the experimental animals and changed every two weeks throughout the duration of the experimental period. The pumps were loaded with 100ul of human IGF1, 50ng/ul. Serum IGF-1 levels were determined with *IGFI By Extraction* (Nichols Institute, CA) twice during the 10 week experimental period.

Northern Analysis for IGFR

Please see specific details in Specific Aim I.

RT-PCR Assay for IGF1, IGFR and IGF2

See specific details in Specific Aim I.

RNA Protection Assay

See specific details in Specific Aim I.

2.1 METHODS Specific Aim III

<u>Specific Aim III:</u> To determine the dose of 17-ß estradiol administration critical to tumor engraftment and progression of growth in *scid/scid* mice that have an impaired GH/IGF-1 axis and if exogenous 17-ß estradiol can futher enhance tumor growth in animals administered optimal concentrations IGF-1 and/or rhGH.

In order to achieve this goal *scid/scid lit/lit* mice treated with rhGH or IGF-1 were further subgrouped to recive 17-β estradiol or a placebo pellet.. Estradiol pellets (Innovative Research of America) were implanted into the subcutaneous tissue of the posterior neck with a trochar. Time to the development of a palpable tumor mass and tumor volume was measured with Vernier calipers and measured as described above. IGF-I and IGF-R levels in experimental tumors is determined by northern and western analyses and compared to levels obtained from *scid/scid lit/lit* mice +/- 17-ß estradiol not receiving rhGH or IGF-1 supplementation.

Northern Analysis for IFG1R

Please see detailed procedures in Specific Aim I.

RT-PCR Assay for IGF1, IGFR and IGF2

See detailed procedures in Specific Aim I.

RNA Protection Assay

See detailed procedures in Specific Aim I.

2.1 METHODS: Specific Aim IV

<u>Specific Aim 4:</u> To grow primary breast cancer explants in the optimized animal model.

This experimental aim will be explored during Year II of this research project. The goal of this experimental aim is to demonstrate that primary human breast cancer explants can be grown and sustained in the optimized animal model developed in Aims 1-3. Human breast carcinomas are obtained from patients undergoing surgery in the operating suites at the Maine Medical Center and are immediately transferred to the laboratory in Earle's minimal essential medium (MEM) for processing. Samples from each tumor are retained for routine pathologic analysis at Maine Medical Center. In addition, specific notation is made of primary tumor size, nuclear grade, axillary lymph node status, the presence or absence of estrogen and progesterone receptors, ploidy and S-phase analysis (this information is readily available after routine

pathologic analysis of the tumor at Maine Medical Center). The tumor is dissected free of necrotic tissue and 2 X 2 mm tumor chunks are cut with a clean scalpel. Experimental animals are anesthetized with 600 ul intraperitoneal injection of Avertin (1.6 gm tribromoethanol/ml tetriary amyl alcohol in 80 ml sterile saline). Under sterile conditions, an incision is made in the skin of the chest wall. A tumor chunk is carefully placed in the region of the mammary fat pad. The incision is closed with Clay Adams staples. One week after surgery staples are removed. Animals are checked twice weekly for any evidence of primary tumor engraftmentment and growth. Tumor measurements and tumor volumes will be scored as described in Specific Aim I.

2.2 RESULTS

Specific Aims IA and III: Growth of MCF7R cells in *scid/scid* mice with or without bolus rhGH and 17 β estradiol.

Table 1: Tumor measurements in NOD *scid/scid* mice exposed to bolus rhGH and/or 17-β estradiol

Figure I: The effect of bolus rhGH and 17-β estradiol on MCF7R tumor cell engraftment and growth in NOD *scid/scid* mice

Table II: Tumor measurements in TghGH *scid/scid* mice

Figure II: MCF7R tumor cell engraftment and growth in TghGH *scid/scid* mice

Table III: Tumor measurements in *scid/scid lit/lit* mice exposed to bolus rhGH and/or 17-β estradiol

Figure III: The effect of bolus rhGH and 17-β estradiol on MCF7R tumor cell engraftment and growth in *scid/scid lit/lit* mice

Table IV: Tumor measurements in *scid/scid lit+/-* mice exposed to bolus rhGH and/or 17-β estradiol

Figure IV: The effect of bolus rhGH and 17-β estradiol on MCF7R tumor cell engraftment and growth in *scid/scid lit+/-* mice

These Tables and Figures are posted and the end of the References section.

Specific Aims 1B and III: Growth of MCF7R cells in *scid/scid* mice with or without continuous infusion rhGH and 17-β estradiol.

Table V: Tumor measurements in NOD *scid/scid* mice exposed to continuous infusion rhGH and/or 17-β estradiol

Figure V: The effect of continuous infusion rhGH and 17-β estradiol on MCF7R tumor cell engraftment and growth in NOD *scid/scid* mice

Table VII: Tumor measurements in *scid/scid lit/lit* mice mice exposed to continuous infusion rhGH and/or 17-β estradiol

Figure VII: The effect of continuous infusion rhGH and 17-β estradiol on MCF7R tumor cell engraftment and growth in *scid/scid lit/lit* mice

Table VIII: Tumor measurements *in scid/scid lit+/-* mice mice exposed to continuous infusion rhGH and/or 17-β estradiol

Figure VIII: The effect of continuous infusion rhGH and 17-β estradiol on MCF7R tumor cell engraftment and growth in *scid/scid lit+/-* mice

These Tables and Figures are posted and the end of the References section. Please note that there is no Table VI. This is intentional. Thank-you.

Specific Aims 1I and III: Growth of MCF7R cells in *scid/scid* mice with or without continuous infusion human IGF1 and 17-β estradiol.

Table 1X: Tumor measurements in NOD *scid/scid* mice mice exposed to continuous infusion human IGF1 and/or 17-β estradiol

Figure IX: The effect of continuous infusion human IGF1 and 17-β estradiol on MCF7R tumor cell engraftment and growth in NOD *scid/scid* mice

Table X: Tumor measurements in *scid/scid lit/lit* mice exposed to continuous infusion human IGF1 and/or 17-β estradiol

Figure X: The effect of continuous infusion human IGF1 and 17-β estradiol on MCF7R tumor cell engraftment and growth in *scid/scid lit/lit* mice

Table XI: Tumor measurements in *scid/scid* lit+/- mice exposed to continuous infusion human IGF1 and/or 17-β estradiol

Figure XI: The effect of continuous infusion human IGF1 and 17-β estradiol on MCF7R tumor cell engraftment and growth in *scid/scid lit+/-* mice

These Tables and Figures are posted and the end of the References section.

Figure XII: Nested RT-PCR assay for IGF-1 demonstrating amplification of IGF1R from MCF7R tumor cells

Figure XIII: Nested RT-PCR assay for IGF-1 demonstrating amplification of IGF2 from MCF7R tumor cells

Figure XV: Initial attempt to develop and RNA protection assay for IGF1R

2.3 DISCUSSION

Bolus rhGH administration and MCF7Rtumor cell growth *in vivo*: In evaluation of the tumor growth curves displayed in Figures I-IV, 17-β estradiol alone is most efficient in stimulating *in vivo* tumor cell engraftment and growth. When rhGH is given in bolus fashion, it appears to inhibit some of the growth stimulatory effects of 17-β estradiol. This is evident most significantly at 5-9 weeks into this study. These observations suggests that rhGH may be stimulating not only the release of growth stimulatory proteins such as IGF1, but a substance(s) that is growth inhibitory.

When rhGH is given alone to animals, there is some growth advantage over control animals. This could be due to IGF1 induction or induction of another growth stimulatory protein. It however, can not stimulate MCF7R growth as efficiently as 17-β estradiol alone.

In animals that are transgenic for human growth hormone, the average tumor volumes at any given time-point are larger than in NOD *scid/scid*. This becomes most

apparent after approximately 8-9 weeks post tumor cell injection. The increase in average tumor volumes may in fact be directly attributable to the presence of growth hormone or more likely other factors. If this was attributable to growth hormone alone, one would expect NOD *scid/scid* animals supplemented with rhGH to have similar tumor volumes. What is striking in TghGH *scid/scid* mice is that animals supplemented with 17-β estradiol have significantly increased tumor growth in comparison to TghGH *scid/scids* not supplemted with 17-β estradiol. This once again suggests that in this animals model, 17-β estradiol is the more important growth factor involved in tumor engraftment and progression.

In *lit/lit* mice there is lack of endogenous growth hormone releasing hormone (ghrh) therefore little if any endogenous murine growth hormone is synthesized in these animals. The full effect of human growth hormone in the xenograft model should be observed in this animal model. The first observation that is made in this set of experiments is that average tumor volume on any specific week of experimentation is smaller in *lit/lit* animals than in any of the other experimental animals. Significant tumor formation did not occur until week number 8 (contrasted to week 6 in NOD *scid/scid* mice). As in NOD *scid/scid* mice, the animals supplemented with 17-β estradiol only resulted in best MCF7R tumor cell engraftment. With no murine IGF1 available in this experimental animal, this suggests that estrogen alone resulted in the upregulation of tumor-made peptides that resulted in cellular proliferation. Again in this model, the supplementation of rhGH to the experimental animals resulted in the blunting of cellular proliferation induced by 17-β estradiol. Human growth hormone supplemented animals had a modest increase in tumor growth but the statitical significance of this is questionable.

Continuous infusion rhGH administration and MCF7R tumor cell growth *in vivo*: Data displayed in Figures V-VIII documents the growth of MCF7R breast cancers in immunodeficient *scid/scid* mice exposed to rhGH administered by continuous infusion. The growth hormone was administered through an alza miniosmotic pump placed in the subcutaneous tissue of the mouse. It appears that continuous infusion of rhGH results in no signtificant alteration of tumor growth in these animal models in comparison to animals treated in the bolus fashion. Molecular studies that are currently pending will further elucidate if significant changes in IGF1, IGF2 and IGFR occurred amongst the various treatment groups.

Continuous infusion human IGF-1 administration and MCF7Rtumor cell growth *in vivo*: In all three types of experimental *scid/scid* mice, the exogenous administration of human IGF-1 resulted in (1) the development of a primary tumor earlier than in control animals and (2) increased and sustained tumor growth over time until the experiment was concluded at 9 weeks post tumor cell injections. The addition of estrogen to animals receiving IGF1 did not appear to further enhance tumor cell engraftment and growth over IGF1 alone. Clearly, in-vitro observations that have been made by others that identify IGF1 as a mitogen and growth stimulatory protein are evident *in vivo* in these experiements.

RT-PCR assays for IGF1, IGF2 and IGFR: IGF2 and IGFR have been successfully amplified from all tumor tissues studied thus far in experimental Aim I (Figure XII and XIII). Tissues from Aim II and III are awaiting processing and will be studied during this next funding period. For IGF2 and IGFR there appear to be no gross differences in the presence of the growth factor and receptor when animals were exposed to estrogen, rhGH or a combination of the two. For discreet measurements of IGF2 and IGFR levels under the various experimental conditions, the RNA protection assay is in the process of being optimized (Figure XIV). The original plan was to achieve these quantitations through northern or western analysis, however, at least in the IGFR situation, the copy number is too low for detection by northern analysis.

IGF1 thus far has not been successfully amplified from MCF7R control cells from tissue culture or any of the tumor explants studied. This has been documented by others for the MCF7 cell line (27). We will continue to look for IGF1 expression in tumors exposed to the growth factors under investigation in this work, however, based on current results, it is unlikely present or present only in ver low copy number. Whether any of the experimental conditions evaluated in this study have the capacity to alter IGF1 tumor expression will be determined as more tumor specimens are studied.

3.0 CONCLUSIONS

In experiments performed to date, it is questionable as to whether rhGH alone or in conjunction with estrogen has a significant role in the primary development of breast cancer in an animal model or the progression of tumor growth in the animal model. The addition to growth hormone may actually be semi-inhibitory to growth of tumors dependent upon estrogen for growth and maintenance. Molecular studies measuring IGF1, IGF2, and IGFR expression in animals treated with rhGH either by daily bolus or continuous infusion are currently in progress in this laboratory. From these experiments, more may be gleaned about rhGH and its role in the regulation and expression of the tumor IGF1, IGF2 and IGFR.

The administration of human IGF1 to animals injected with MCF7R tumor cells clearly enhances not only the time to development of a palpable primary tumor but also has a role in sustaining tumor growth and size over and above what has been achievable with estrogen alone. Clearly from the *in vivo* data presented here, the presence of human IGF1 may be critical to the successful development of breast cancer xenograft models. The effect of human IGF1 on tumor IGF1, IGF2 and IGFR is currently under evaluation in this laboratory on tumor specimens obtained from the experimental animals. Hopefully these studies will further elucidate IGF1's importance as a growth factor in these animal models.

Over the next year in this laboratory, primary tumors from patients under care at Maine Medical Center, will be place into the *scid/scid* mouse model and supplemented with IGF1 to establish if our preliminary results can be applied to the development of new xenograft models.

The initial statement of work presented to the army for completion of this work is displayed in the appendices to this document. The work will be completed as planned. There have been some delays to quantification of tumor growth factors due to the fact that northern analyses proved to be incapable of accurate quantifications for IGFR.

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TABLE I: Tumor measurements in NOD *scid/scid* mice exposed to bolus rhGH and or 17 beta estradiol

Tumor cells 2 X 10^5 MCF-7R cells injected in mammary fat pad on 2/5/98

Animal	Ear		Average tumor volume in mm^3										
	Animal number		1	2	3	4	5	6	7	8	9	10	11
no estrogen, no growth hormone													
NOD A	0	0	0	0	0.09	0.78	0.78	0.78	6.3	58.9	141	50.2	169
NOB B	1	0	0	0	0	0	0.78	0	6.3	9.4	21.9	6.3	50.2
NOD C	2	0	0	0	0.09	0.09	0.09	6.3	6.3	14.1	98.1	98	6.3
NOD D	3	0	0	0	0.09	0.09	0.78	0.78	9.4	6.3	21.9	21.9	25.2
NOD E	4	0	0	0	0.09	0.09	0.09	0.78	0.78	0.78	6.3	22	98
Average tumor Volum		0	0	0	0.072	0.21	0.504	1.728	5.816	17.896	57.84	39.68	69.74
SD		0	0	0	0.040249	0.321014	0.377929	2.578046	3.118859	23.42725	58.68601	32.4186	58.38053
no estrogen, plus grov		0	1	2	3	4	5	6	7	8	9	10	11
NOD F	0	0	0	0.09	0.09	0.78	0.78	0.78	6.3	6.3	169	307.7	401.9
NOD G	1	0	0	0.09	0.09	0.78	0.78	6.3	25.1	78.5	215.8	307.7	250
NOD H	2	0	0	0.09	0.09	0.78	0.78	153.8	98.1	200.9	381	269	904.3
NOD I	3	0	0	0.09	0.09	0.78	6.3	153.8	50.2	98.1	113	224	141.3
NOD J	4	0	0	0.09	0.09	0.78	6.6	6.3	50.2	29.4	169	282	282
Average tumor Volum		0	0	0.09	0.09	0.78	3.048	64.196	45.98	82.64	209.56	278.08	395.9
SD		0	0	0	0	0	3.107398	81.82792	34.50575	75.67013	102.524	34.55846	298.9932
plus estrogen, plus gr		0	1	2	3	4	5	6	7	8	9	10	11
NOD K	0	0	0	0.09	0.09	6.3	6.3	0.78	98.1	115			
NOD L	1	0	0	0.09	0.09	0.78	0.78	169.6	197	351.7	346	169	401
NOD M	2	0	0	0.09	0.09	6.3	6.3	98.1	98.1	9.4	346	502	1326
NOD N	3	0	0	0.09	0.09	0.78	0	0.78	78.5	78.5	230	572	942
NOD O	4	0	0	0.09	0.09								
Average tumor Volum		0	0	0	0	3.54	3.345	67.315	117.925	138.65	307	163.5556	889.6667
SD		0	0	0	0	3.186973	3.426967	82.18627	53.52024	148.6306	66.97263	215.3284	464.7153
plus estrogen, no grov		0	1	2	3	4	5	6	7	8	9	10	11
NOD P	0	0	6.3	0.09	6.3	98.1	169	269	169	307.7	346	785	785
NOD Q	1	0	0	0.09	6.3	56.5	200.9	471	251.2	445	628	706	1017.3
NOD R	2	0	0	0.09	0.09	6.3	141.3	78.5	98	197	251	401	628
NOD S	3	0	0	0.09	0	0.78	0.78	0.78	0.78	6.3	6.3	508	854
NOD T	4	0	0	0.09	6.3	50.2	113	549	572	445	445	785	1044
Average tumor Volum		0	1.26	0.09	3.798	42.376	124.996	273.656	218.196	280.2	280.8833	637	865.66
SD		0	2.817446	0	3.426152	39.99241	76.70904	238.3945	218.1763	185.0796	245.7128	173.8577	171.7004

FIGURE I: Effect of bolus rhGH and 17 beta estradiol on MCF-7 growth in NOD *scid/scid* mice

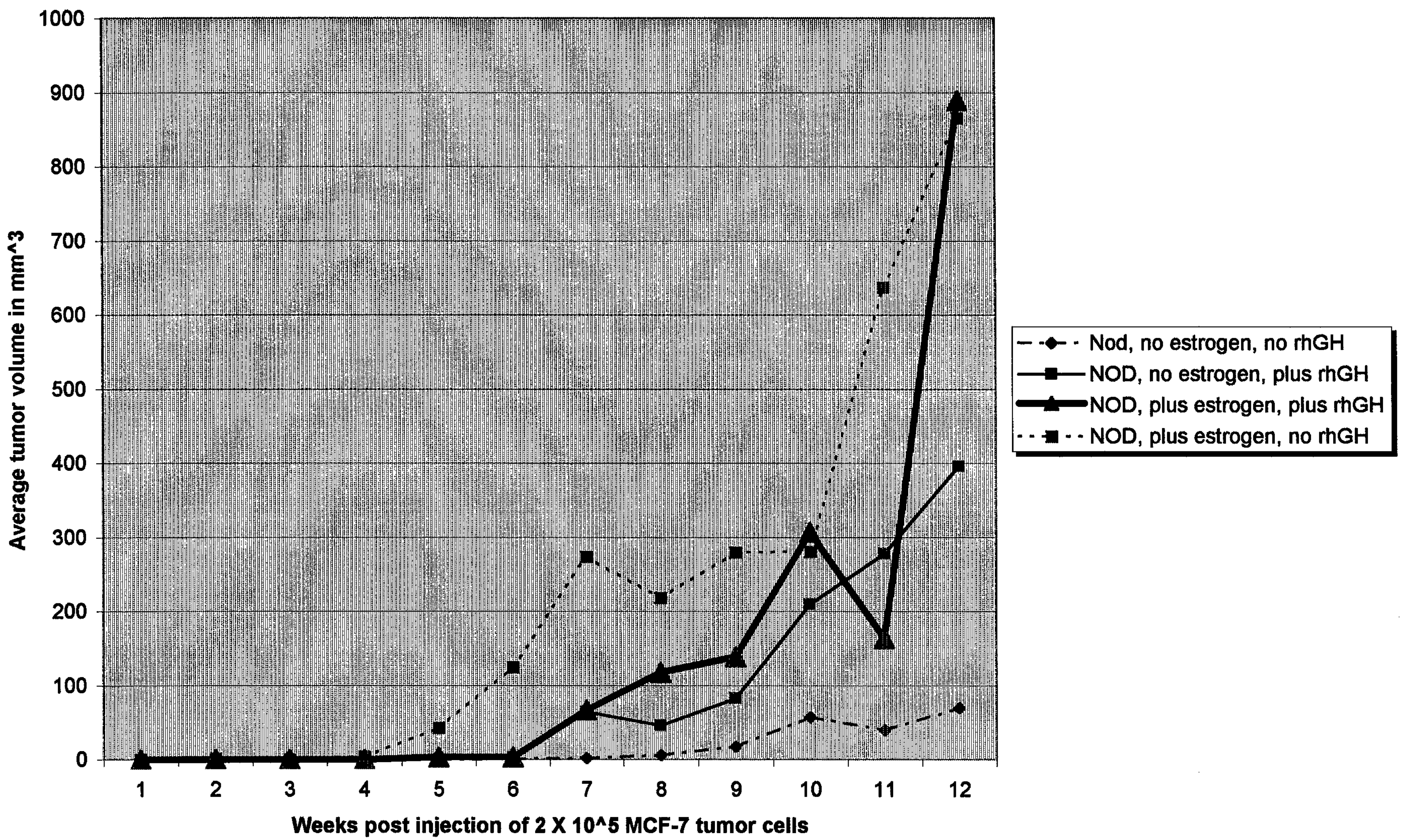


TABLE II: Tumor measurements in TghGH *scid/scid* mice

Tumor cells 2 X 10^5 MCF7R cells injected into the mammary fat pad on 7.15.98

	7/15/98	7/22/98	8/6/98	8/14/98	8/19/98	8/25/98	9/1/98	9/10/98	9/16/98	9/23/98	10/1/98
week											
Animal	0	1	2	3	4	5	6	7	8	9	10
No estrogen											
TghGH scid A	0	palp	4 X3	4X5	6X5	9X5	9X9	9X6	11X11	8X7	8X8
TghGH scid B	0	0	0	palp	palp	palp	palp	palp	palp	3X3	5X5
TghGH scid C	0	0	0	0	0	palp	palp	dead	dead	dead	dead
Plus estrogen											
TghGH scid A	0	0	4X3	5X5	4X4	5X5	7X7	8X7	13X13	13X13	13X13
TghGHscid B	0	0	5X4	6X5	5X5	7X6	8X8	7X7	7X12	10X12	15X10
TghGH scid C	0	0	0	palp	palp	palp	7X7	10X5	15X10	15X10	dead
TghGHscid D	0	0	2X2	3X5	3 X4	9X9	9X9	dead	dead	dead	dead
Animal											
No estrogen											
TghGH scid A	0	0.78	37.7	31.4	141	317	572	381	1044	351	401
TghGH scid B	0	0	0	0.78	0.78	0.78	0.78	0.78	0.78	21.2	98
TghGH scid C	0	0	0	0	0	0.78	0.78	dead	dead	dead	dead
Average tumor volume	0	0.26	12.56667	10.72667	47.26	106.1867	191.1867	190.89	522.39	186.1	249.5
Standard deviation		0.450333	21.76611	17.90788	81.18216	140.5422	253.8756	190.11	521.61	164.9	151.5
Plus estrogen											
TghGH scid A	0	0	37.7	98.1	50.2	98.1	269	351	1724	1724	1724
TghGHscid B	0	0	78.5	141	98.1	230	401.9	269	461	942	1766
TghGH scid C	0	0	0	0.78	0.78	0.78	85.8	392	1766	1766	dead
TghGHscid D	0	0	6.3	35.3	28.2	572	572	dead	dead	dead	dead
Average tumor volume	0	0	30.625	68.795	44.32	225.22	332.175	337.3333	1317	1477.333	1745
standard deviation			35.92431	62.77022	41.16005	175.78	154.775	45.55556	570.6667	356.8889	21

FIGURE II: MCF7R tumor growth in TghGH *scid/scid* mice

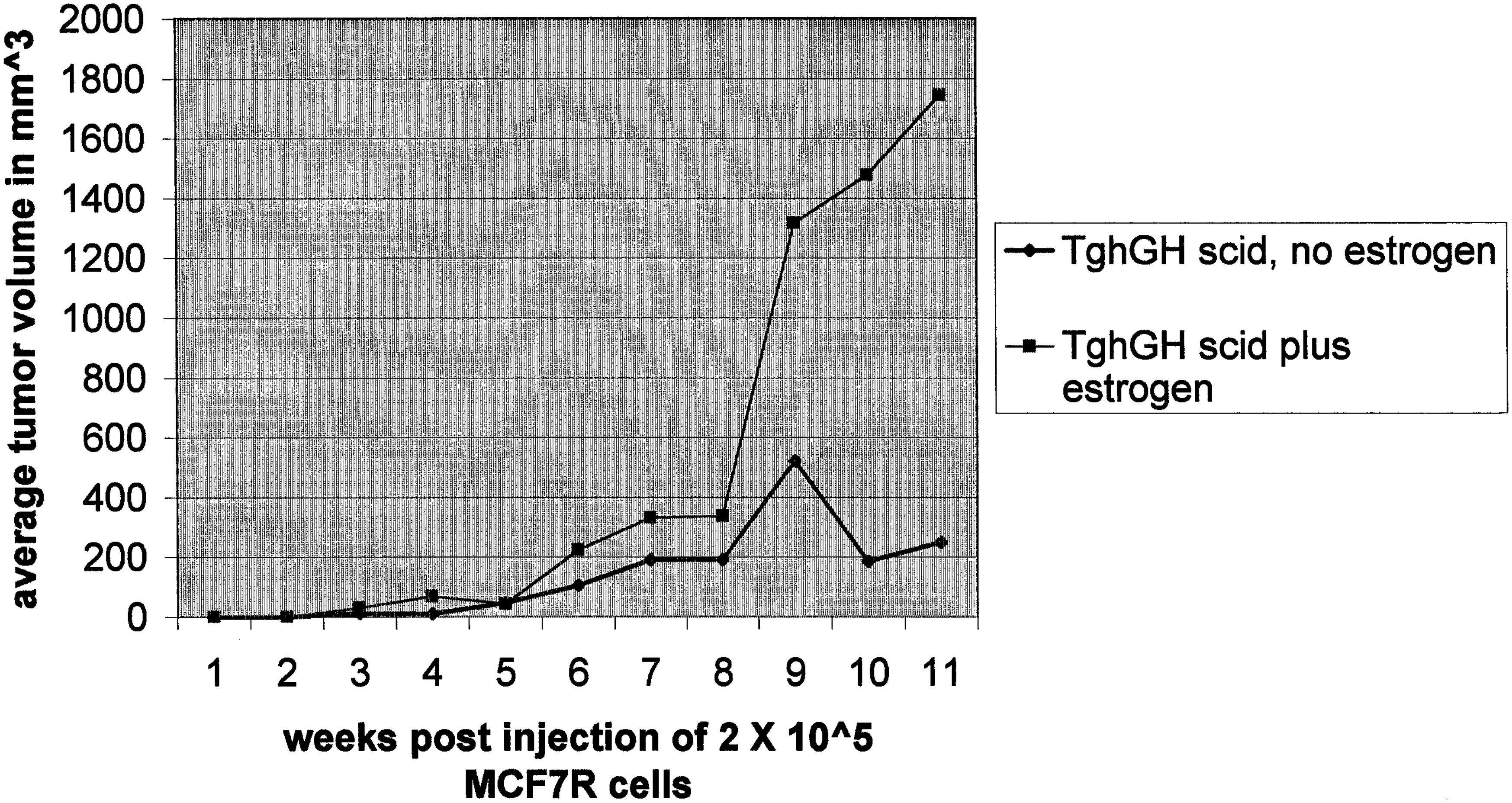


TABLE III: Tumor measurements in *scid/scid lit/lit* mice exposed to bolus rhGH and/or 17 beta estradiol

Tumor cells 2 X 10^5 MCF-7R cells injected in mammary fat pad on 2/5/98

Animal	Ear Animal numbei	Tumor size in mm^3 0	1	2	3	4	5	6	7	8	9	10	11
no estrogen, no growth hormone													
LIT A	0	0	0	0	6.3	6.3	6.3	21.2	0.78	0.78	58	141	157
LIT B	1	0	0	0	0	0	0	0	0.78	0.09	0.78	78.5	317
LIT C	2	0	0	0	0	0.78	21.2	0	0	0	0	0.78	0.78
LIT D	3	0	0	0	0	0	0	0.78	6.3	141.3	98	117.7	678
LIT E	4	0	0	0	0.78	0.78	0.78	0.78	98.1	98.1	98	12.5	307
Average tumor Volume		0	0	0	1.416	1.572	5.656	4.552	21.192	48.054	50.956	70.096	291.956
SD		0	0	0	2.751051	2.671651	9.080015	9.314683	43.06684	67.16394	48.96437	62.22168	251.4359
no estrogen, plus growth hormone													
LIT F	0	0	0	0	0.09	0.78	0.78	0.78	9.4	78.5	137	98.1	169.6
LIT G	1	0	0	0	0.09	0.78	0.78	0.78	62.8	78.5	78.5	141.3	78.5
LIT H	2	0	0	0	0	0	0	25.1	0.09	113	78.5	58.9	230.8
LIT I	3	0	0	0	0	0	0.78	0.78	0.78	21.2	230.8	141	98.1
LIT J	4	0	0	0	0	0	0	0.78	0.78	6.3	78.5	98.1	307
Average tumor Volume		0	0	0	0.036	0.312	0.468	5.644	14.77	59.5	120.66	107.48	176.8
SD		0	0	0	0.049295	0.427224	0.427224	10.87623	27.12315	44.38857	66.57742	34.65317	94.57333
plus estrogen, plus growth hormone													
LIT K	0	0	0	0	0	0.78	0.78	0.78	0.78				
LiIT L	1	0	0	0	0.78	0.78	6.3	6.3	98.1	137	254.3	226	346.2
LIT M		0	0	0						0.78	87.9	117.7	141.3
LIT N	2	0	0	0	0.78	0.78	0.78	0.78	9.4	6.3	50.2	269	269.2
LIT O	3	0	0.09	0.09	14.1	0.78	25.1	37.6	98.1	78.5	98.1	141.3	307.7
Average tumor Volume		0	0.018	0.018	3.915	0.078	8.24	11.365	51.595	55.645	122.625	188.5	266.1
SD		0	0.040249	0.040249	6.799949	0	11.53728	17.68251	53.81453	64.77161	90.16834	71.00934	88.94047
plus estrogen, no growth hormone													
LIT P	0	0	0	0	0.09	0.78	0	0.78	9.4	192.3	301.4	301.4	351.7
LIT Q	1	0	0	0	25.1	37.6	37.6	37.6	37.6	117.7	230.8	85.8	85.8
LIT R	2	0	0	0	21.2	0.78	0	0	0	0	0	169.6	169.6
LIT S	3	0	0	0	0.09	0.78	0.78	0.78	230.8	200.9	351.8	301.4	351.7
LIT T	4	0	0	0	0	0	0	0.78	0.78	169.6	50.2	0.78	6.3
Average tumor Volume		0	0	0	9.296	7.988	7.676	7.988	55.716	136.1	186.84	171.796	193.02
SD		0	0	0	12.72191	16.55706	16.73143	16.55706	99.05807	82.67633	154.7966	132.5151	155.9388

FIGURE III: The effect of bolus rhGH and 17 beta estradiol on MCF-7 tumor cell engraftment in *scid/scid lit/lit* mice

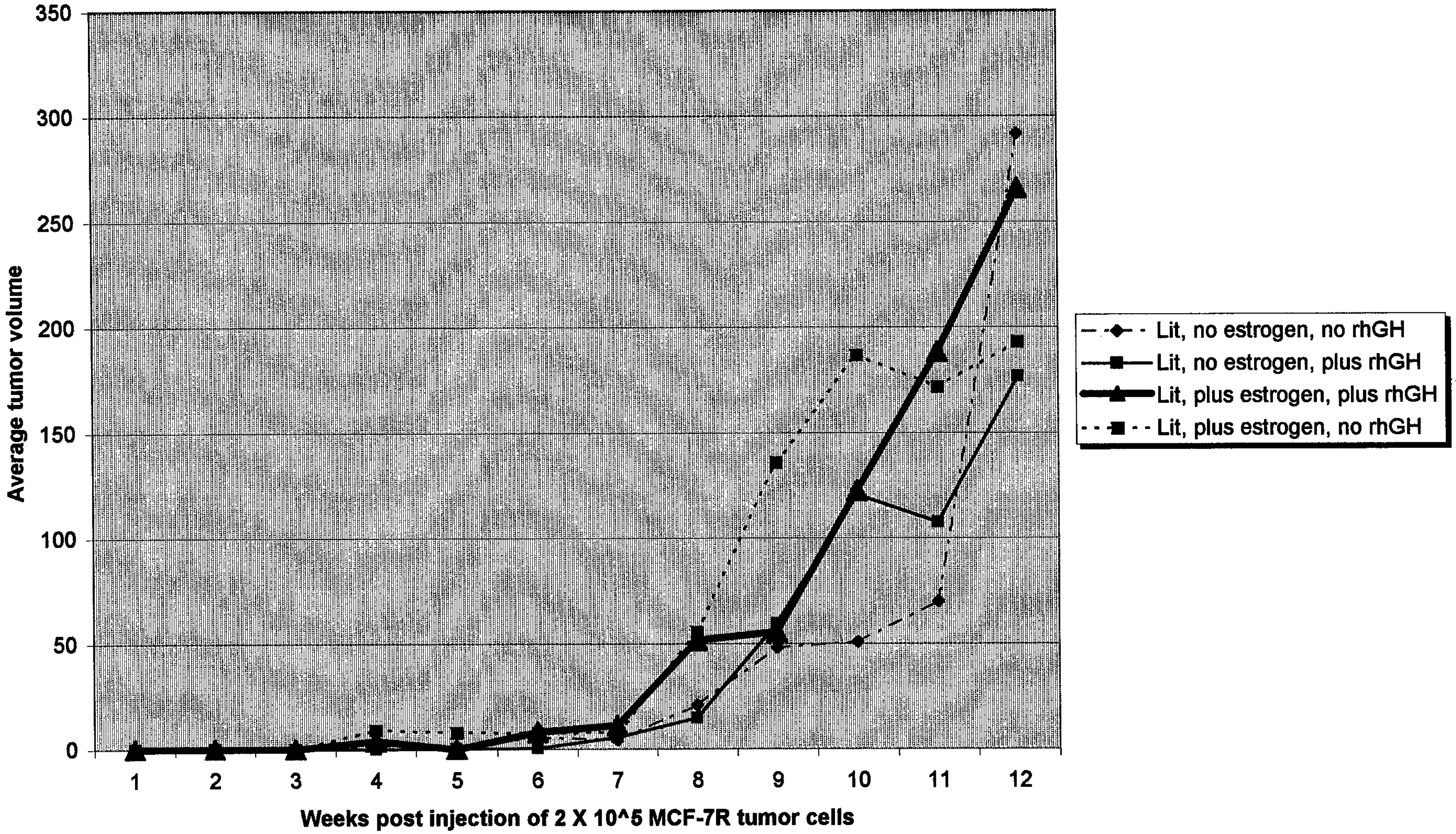


TABLE IV: Tumor measurements in *scid/scid lit+/-* mice exposed to bolus rhGH and/or 17 beta estradiol

Tumor cells 2 X 10^5 MCF-7R cells injected in mammary fat pad on 2/5/98

Animal	Tumor volumes in mm^3											
	0	1	2	3	4	5	6	7	8	9	10	11
no estrogen, no growth hormone												
LIT +/- A	0	0	0	0	0	0	6.3	21.2	78.5	62.8	226	452
LIT +/- B	0	0	0	0.09	0.78	0	6.3	6.3	28.3	35.3	904	904
LIT +/- C	0	0	0	0.09	0.78	0.78	6.3	6.3	21.2	58.9	169	269
LIT +/- D	0	0	0	0.09	0.78	0	0	6.3	9.4	21.2	6.3	6.3
LIT +/- E	0	0	0	0	0	0	6.3	0	0.09	0.78	98.1	98.1
LIT +/- Z	0	0	0	0	0	0	0	0.78	0.78	0.78	269	351
Average tu	0	0	0	0.045	0.39	0.13	4.2	6.813333	23.045	29.96	278.7333	346.7333
SD	0	0	0	0.049295	0.427224	0.318434	4.2	7.623508	29.37728	27.29171	320.2261	317.9312
no estrogen, plus growth hormone												
LIT +/- F	0	0	0.09	0.09	0.78	0.78	6.3	0.078	9.4	21.2	301	785
LIT +/- G	0	0	0.09	0.09	0.78	0.78	6.3	6.3	9.4	78.5	6.3	21.2
LIT +/- H	0	0	0	0.09	0.78	0.78	0.78	35.3	251.2	169.6	200.96	549
LIT +/- I	0	0	0	0.09	0.78	0.78	0.78	0.78	113	192.3	200.9	98.1
LIT +/- J	0	0	0	0.09	0.78	6.3	9.4	0	78.5	192.3	301	301
LIT +/- Y	0	0	0	0	0	0	0	6.3	0.09	0.09	0.09	0.09
Average tu	0	0	0.03	0.075	0.65	1.57	3.926667	8.126333	76.93167	108.9983	168.375	292.3983
SD	0	0	0.046476	0.036742	0.290689	2.338127	3.910103	13.63716	96.64215	87.23602	135.563	317.9054
plus estrogen, plus growth hormone												
LIT +/- K	0	0.09	9.4	21.2	62.8	48.98.1	200.9	192.3	317.9			
LIT +/- L	0	0.09	0.09	0.09						381	471	904
LIT +/- M	0	0	0.09	14.1	78.5	39.2	78.5	169.6	141.3	251.2	98.1	445
LIT +/- N	0	0	0.09	0.09	0.78	0.78	141.3	153.9	98.1	169.6	200.9	98.1
LIT +/- O	0	0	0.09	0.09	6.3	0.78	230.8	169.6	381.5	269	401.9	452
Average tu	0	0.036	1.952	7.114	37.095	13.58667	162.875	171.35	234.7	267.7	292.975	474.775
SD	0	0.049295	4.163559	9.940188	39.33714	22.1818	67.43967	15.80643	136.4497	87.05255	173.2163	330.4156
plus estrogen, no growth hormone												
LIT +/- P	0	0	9	0.09	0.78	0	78.5	0.78	0.78	502	269	401
LIT +/- Q	0	0	0.09	0.09	0.78	0.78	98.1	98.1	192.3	254	98.1	98.1
LIT +/- R	0	0	0	6.3	6.3	0.78	113	62.8	98.1	141.3	98.1	21.2
LIT +/- S	0	0	0	0.09	0.78	0	9.4	9.4	141.3	269	230	269
Average tu	0	0	2.2725	1.6425	2.16	0.39	74.75	42.77	108.12	291.575	173.8	197.325
SD	0	0	4.485201	3.105	2.76	0.450333	45.80018	45.96856	81.25979	151.4186	88.84905	170.7693

FIGURE IV: The effect of rhGH and 17 beta estradiol on MCF-7 R tumor cell engraftment in *lit* +/- *scid/scid* mice

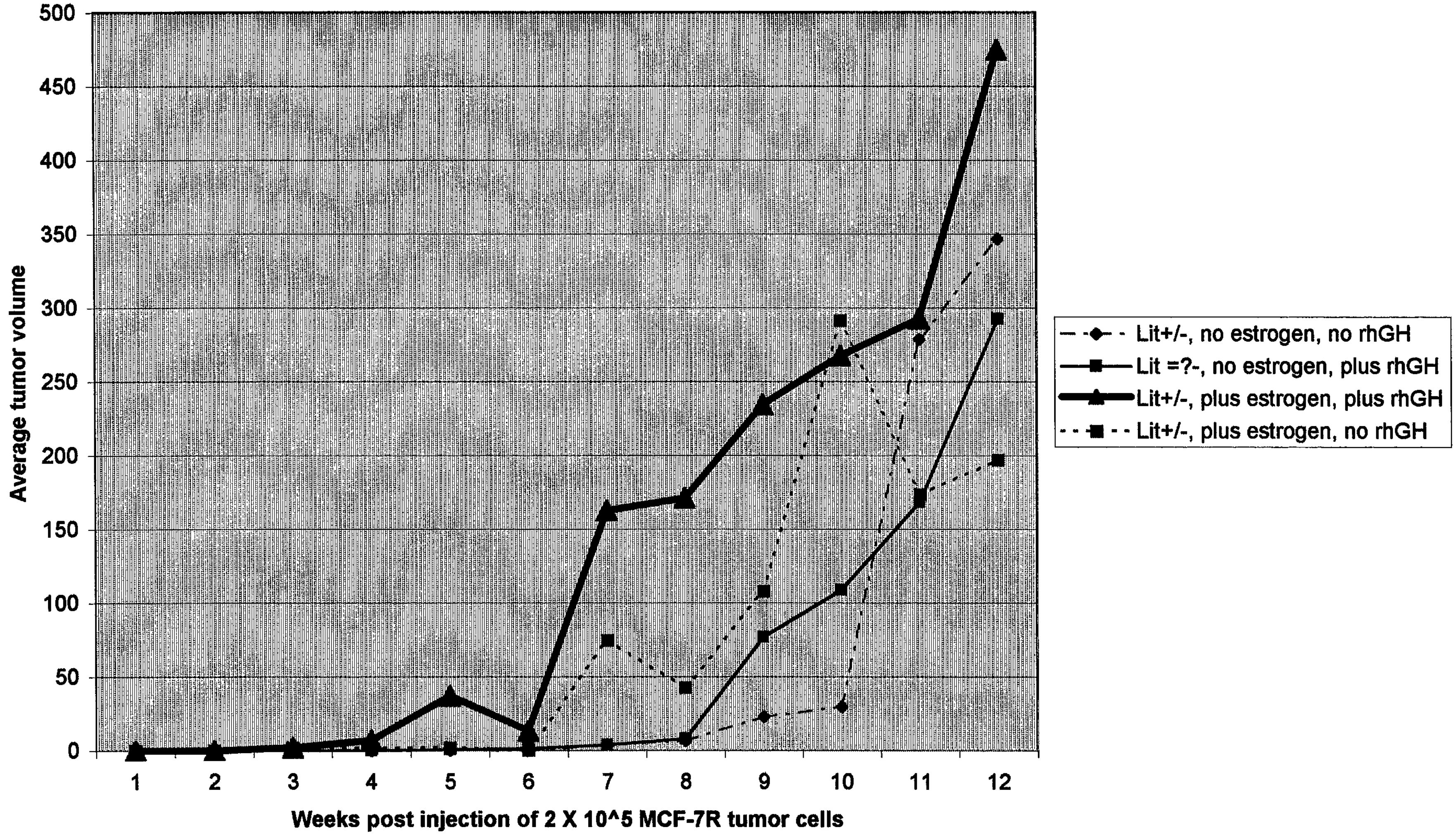


TABLE V: Tumor measurements in NOD scid/scid mice exposed to continuous infusion rhGH and/or 17 beta estradiol

Tumor cells 2 X 10^5 MCF-7 R cells injected in mammary fat pad 4/17/98

	28-Apr	5-May	12-May	21-May	28-May	2-Jun	6/10/98	6/17/98	6/24/98	7/1/98	7/7/98
Animal	Weeks post injection										
no estrogen, no GH	1	2	3	4	5	6	7	8	9	10	11
NODA	0	0	0	0	0	0	0	0.78	6.28	98	98
NODB	0	0	0	0	0	0	0	6.28	50.24	230	381.5
NODC	0	0	0	0.78	0.78	0.78	0.78	21.2	98.1	230	445
NOD C2	0	0	0	0	0.78	0.78	0.78	78.5	98.1	635	452
Average Tumor Volume	0	0	0	0.195	0.39	0.43	0.43	26.69	63.18	168.375	344.125
Standard Deviation	0	0	0	0.39	0.45033321	0.450333	0.450333	35.60109	44.13565	232.9641	167.1199
no estrogen, plus GH											
NOD D	0	0.78	0.78	0.78	0.78	0.78	0.78	37.7	37.6	346	423
NOD E	0	0.78	0.78	0.78	0.78	153.8	6.5	137.4	169	226	137
NOD F	0	0	0	0	0.78	98.1	0.78	98.1	78.5	452	572
NOD G	0	0	0	0							
Average Tumor Volume	0	0.39	0.39	0.39	0.78	84.22667	2.686667	91.06667	95.03333	341.3333	377.3333
Standard Deviation	0	0.45033321	0.39	0.450333	1.49012E-08	77.44761	3.302444	50.22075	67.24212	113.0722	221.0664
plus estrogen, plus GH											
NOD H	0	0.78	6.3	6.3	98.1	197	169	141.3	214	282	282
NOD I	0	0.78	0.78	0.78	6.3	98.1	6.3	35.3	392	628	942
NOD J	0	0.78	0.78	0.78	0.78	141.3	0.78	62.8	452	635	1017
Average tumor Volume	0	0.78	2.62	2.62	35.06	145.4667	58.69333	79.8	352.6667	515	747
Standard Deviation	0	1.49012E-08	3.186973	3.186973	54.66396253	49.58148	95.56824	55.00682	123.7794	201.8143	404.4441
plus estrogen, no GH											
NOD K	0	0	0.78	0.78	21.9	78.5	117.7	197.8	549	1031	863
NOD L	0	0	0.78	0.78	50.9	192.3	226	226	182	502	863
NOD M	0	0	0	31.9	21.9	78.5	153	381.5	445	502	785
NOD N	0	0	0	50.9	113	192.3	169	307	351	269	452
Average tumor volume	0	0	0.39	21.09	51.925	135.4	166.425	278.075	381.75	576	740.75
Standard Deviation	0	0	0.450333	24.70145	42.95038805	65.70246	45.13006	83.0447	155.7977	322.6071	195.9802

FIGURE V:

Effect of continuous infusion rhGH and 17 beta estradiol on MCF-7R growth in NOD scid mice

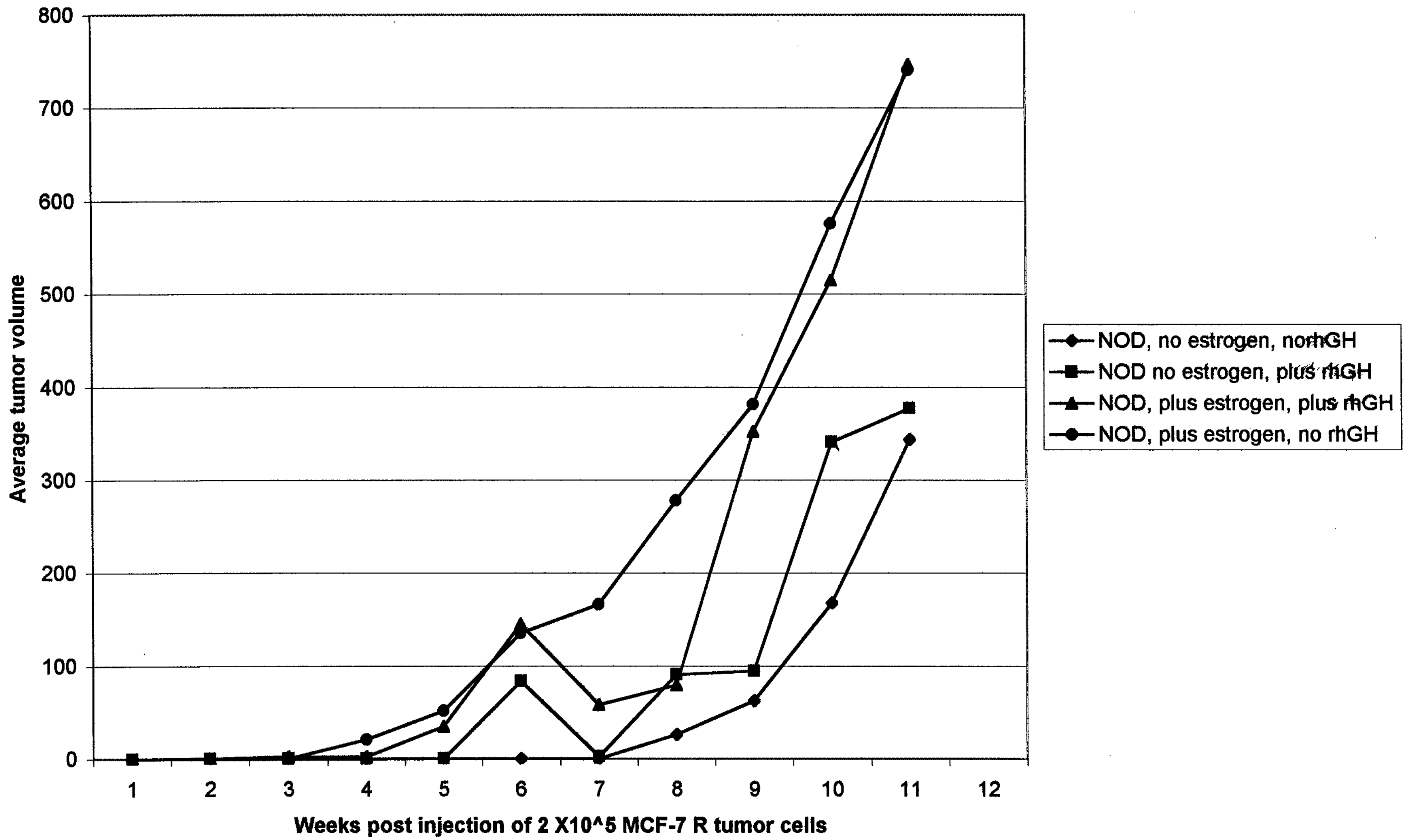


TABLE VII: Tumor measurement in scid/scid lit/lit mice exposed to continuous infusion rhGH and/or 17 beta estradiol

Tumor cells 2 X 10^5 MCF-7 R cells injected in mammary fat pad 4/17/98

	28-Apr	5-May	12-May	21-May	28-May	2-Jun	6/10/98	6/17/98	6/24/98	7/1/98	7/7/98
Animal	Weeks post injection										
no estrogen, no GH	1	2	3	4	5	6	7	8	9	10	11
lit A	0	0	0	0	0	0	0.09	0.78	6.28	169	98
lit B	0	0	0	0	0.78	0.78	0.78	9.4	98.1	62.8	98
lit C	0	0	0	0.78	0.78	0.78	0.78	25.1	21	21	21
lit D	0	0	0	0.78	6.3	6.3	6.3	37.7	21	230	21
average tumor volume	0	0	0	1.112	1.965	1.965	1.9875	18.245	36.595	98.56	49.8
Standard deviation	0	0	0	0.450333	2.913297	2.913297	2.893341	16.41898	41.58635	96.66369	44.18937
no estrogen, plus GH											
lit E	0	0.78	0.78	50.2	40.9	98.1	137.4	226	113	197	269
lit F	0	0.78	0.78	50.2	28.5	98.1	113	269	230	549	401
lit G	0	0.78	0.78	21.9	21.9	78.5	98.1	251	141	197	269
lit H	0	0.78	0.78	21.9	6.3	21.9	78.5	169.6	197	452	502
lit I	0	0	0	6.3	6.3	0.78	78.5	230.8	502	863	401
average tumor volume	0	0.624	0.624	30.1	20.78	59.476	101.1	229.28	236.6	451.6	368.4
Standard deviation	0	0.348827	0.348827	19.42254	14.87454	45.28442	24.94905	37.49736	155.2942	277.6595	99.66845
plus estrogen, plus GH											
litJ	0	0.78	0.09	6.3	21.2	197	197	269	346	346	384
lit K	0	21.9	0.09	0	0.78	37	78.5	197	230	269	226
lit L	0	6.3	0.78	0.78	21.9	0.78	9.4	28	137	37	58
Average tumor volume	0	9.66	0.32	2.36	14.62667	78.26	94.96667	164.6667	237.6667	217.3333	222.6667
Standard deviation	0	10.95357	0.398372	3.434356	11.99667	104.4144	94.87783	123.7107	104.7107	160.8488	163.0256
plus estrogen, no GH											
lit M	0	0.78	0.78	6.3	21.9	6.3	6.3	137.4	192	854	653
lit N	0	0	0.78	6.3	21.9	98.1	98.1	141.3	230.7	635	502
lit O	0	0	0.78	6.3	21.9	98.1	117	195	141	301	384
lit P	0	0	0.78	50.9	37.7	98.1	169	269	269	226	98
Average tumor volume	0	0.195	0.78	17.45	25.85	75.15	97.6	185.675	208.175	504	409.25
Standard deviation	0	0.39	0	22.3	7.9	45.9	67.84851	61.45358	54.71492	293.3451	234.8977

FIGURE VII:

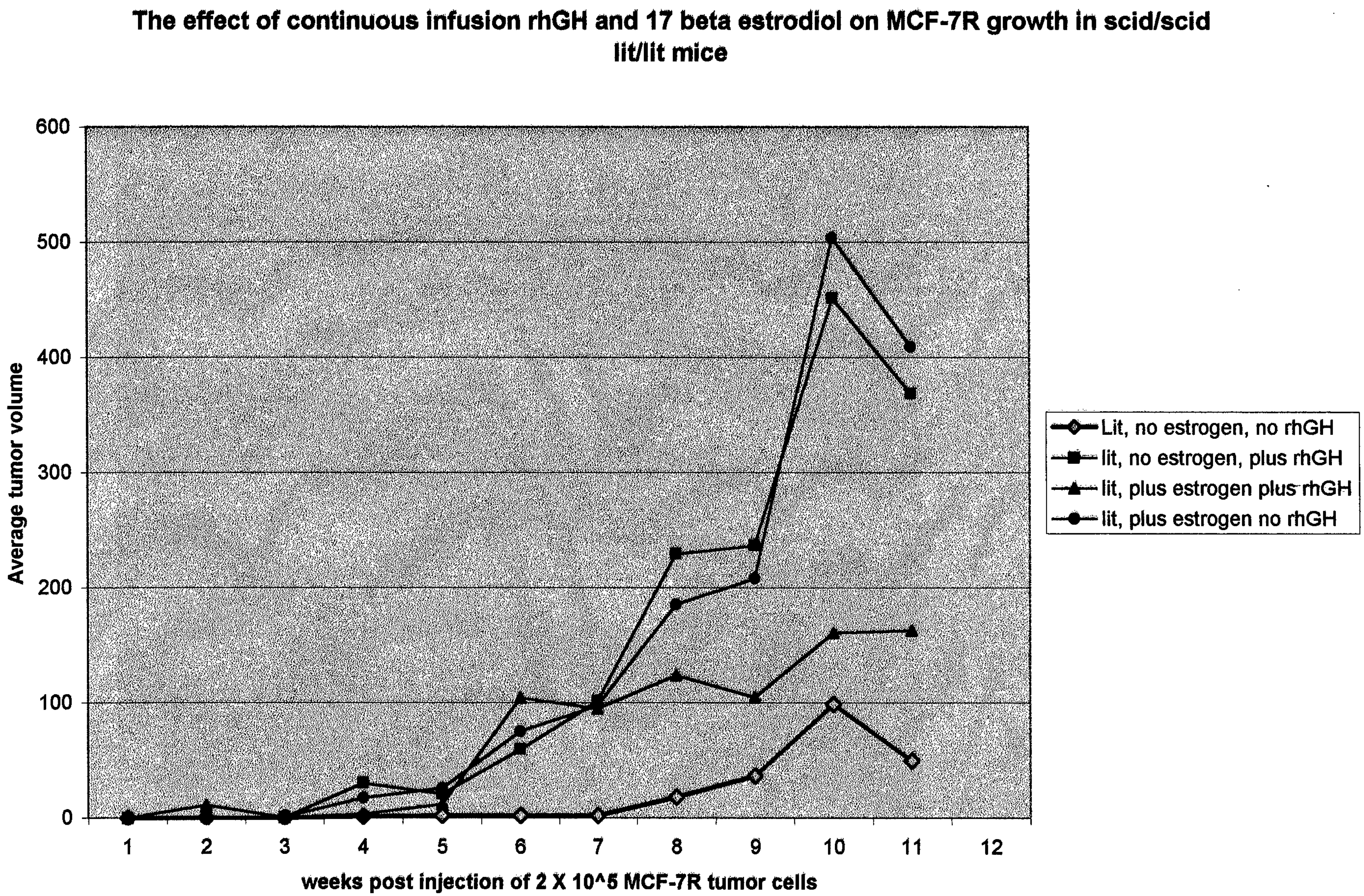
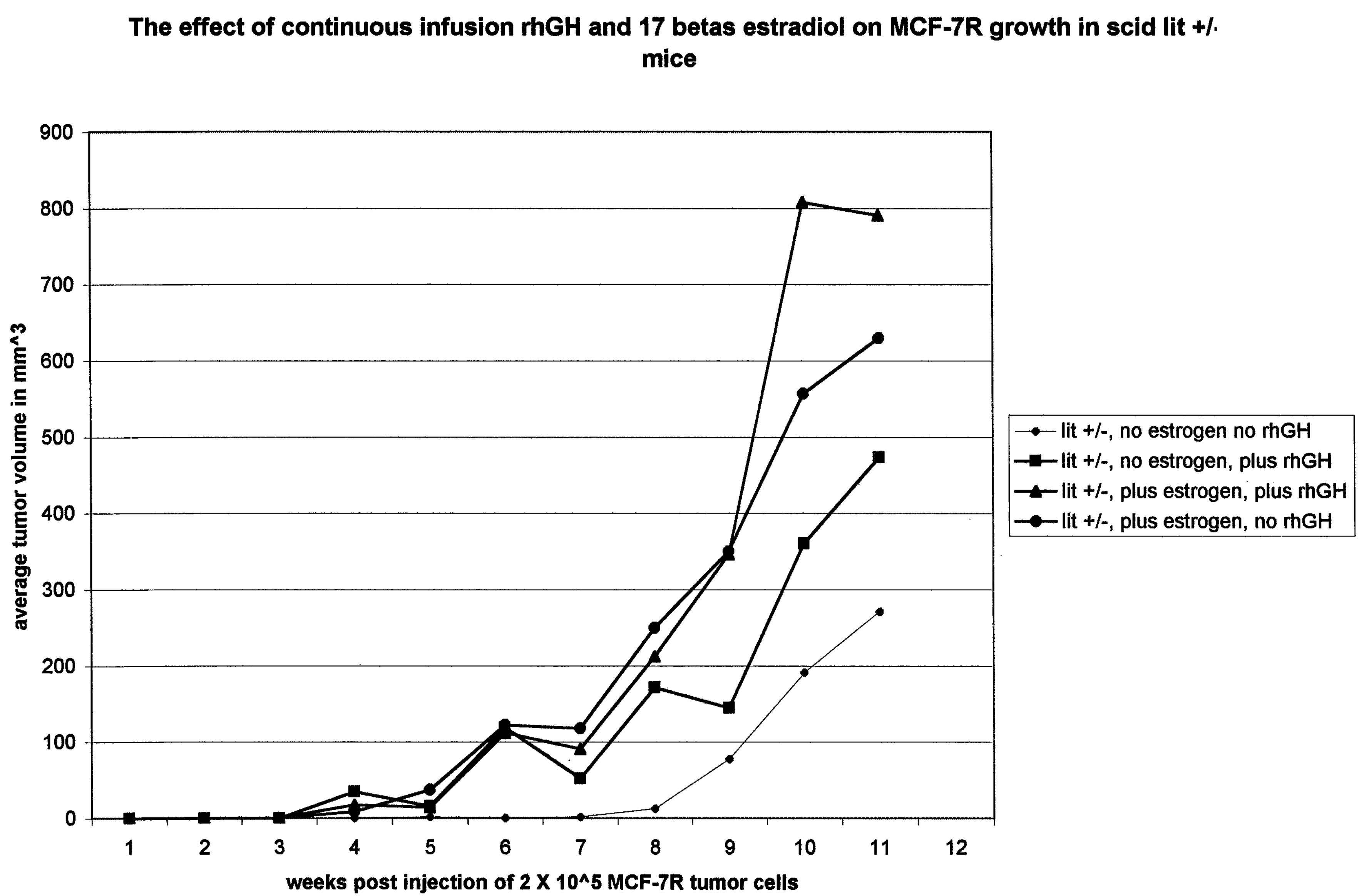

The effect of continuous infusion rhGH and 17 beta estrodiol on MCF-7R growth in scid/scid lit/lit mice
Average tumor volume
600
500
400
300
200
100
0
1
2
3
4
5
6
7
8
9
10
11
12
weeks post injection of 2 X 10^5 MCF-7R tumor cells
Lit, no estrogen, no rhGH
lit, no estrogen, plus rhGH
lit, plus estrogen plus rhGH
lit, plus estrogen no rhGH

TABLE VIII: Tumor measurements in scid/scid lit +/- mice exposed to continuous infusion rhGH and/or 17 beta estradiol

Tumor cells 2 X 10^5 MCF-7 R cells injected in mammary fat pad 4/17/98

	28-Apr	5-May	12-May	21-May	28-May	2-Jun	6/10/98	6/17/98	6/24/98	7/1/98	7/7/98
Animal	Weeks post injection										
no estrogen, no GH	1	2	3	4	5	6	7	8	9	10	11
lit +/- A	0	0	0	0	0	0	0	0			
lit +/- B	0	0	0	0	0	0.78	0	6.3	98	269	401
lit +/- C	0	0	0	0.78	0.78	0.78	0.78	6.3	37.7	137	215
lit +/- D	0	0	0	0.78	6.3	0.78	6.3	37.7	98	169	197
average tumor volume	0	0	0	0.39	1.77	0.585	1.77	12.575	77.9	191.6667	271
Standard deviation	0	0	0	0.450333	3.042302	0.39	3.042302	17.01125	34.81422	68.85734	112.9425
no estrogen, plus GH											
lit +/- E	0	0.78	0.78	25.1	25.1	98.1	6.3	230.8	192	452	502
lit +/- F	0	0	0	78.5	6.7	141	98.1	113	98	269	445
lit +/- G	0	0	0	0.78							
average tumor volume	0	0.26	0.26	34.79333	15.9	119.55	52.2	171.9	145	360.5	473.5
Standard deviation	0	0.450333	0.450333	39.75638	13.01076	30.33488	64.9124	83.29718	66.46804	129.4005	40.30509
plus estrogen, plus GH											
lit +/- H	0	0.78	0.78	21.9	21.9	141.3	98.1	137.4	254	863	653
lit +/- I	0	0.78	0.78	21.9	21.9	98.1	98.1	230.8	401	699	699
lit +/- J	0	0	0.78	21.9	0.78	98.1	78.5	269.3	384	863	1020.5
lit +/- K	0	0	0.78	6.3							
average tumor volume	0	0.39	0.78	18	14.86	112.5	91.56667	212.5	346.3333	808.3333	790.8333
Standard deviation	0	0.450333	0	7.8	12.19364	24.94153	11.31607	67.8275	80.41351	94.68544	200.2226
plus estrogen, no GH											
lit +/- L	0	0	0.78	6.3	21.9	98.1	137	346	552	502	635
lit +/- M	0	0	0.78	6.3	49.9	197.8	166	254	502	942	942
lit +/- N	0	0	0.78	21.9	78.5	192.3	169	401	346	785	942
lit +/- O	0	0	0	0.78	0.78	0.78	0.78	0.78	0.78	0	0
average tumor volume	0	0	0.585	8.82	37.77	122.245	118.195	250.445	350.195	557.25	629.75
Standard deviation	0	0	0.39	9.099978	33.79441	93.01073	79.5956	177.1467	248.9166	413.716	444.0769
						162.7333	157.3333	333.6667	466.6667	743	839.[illegible]
						56.04162	17.67295	74.27202	107.4492	222.9865	177.2[illegible]

FIGURE VIII:



The effect of continuous infusion rhGH and 17 betas estradiol on MCF-7R growth in scid lit +/- mice
average tumor volume in mm^3
0
100
200
300
400
500
600
700
800
900
1
2
3
4
5
6
7
8
9
10
11
12
weeks post injection of 2 X 10^5 MCF-7R tumor cells
lit +/-, no estrogen no rhGH
lit +/-, no estrogen, plus rhGH
lit +/-, plus estrogen, plus rhGH
lit +/-, plus estrogen, no rhGH

TABLE IX: Tumor Measurements in NOD *scid/scid* mice exposed to continuous infusion IGF1 and/or

Tumor cells 2 X 10^5 MCF-7R cells injected into the mammary fat pad on 7/29/98

	Weeks post injection of tumor cells								
Animal	8/6/98	8/13/98	8/19/98	8/25/98	9/1/98	9/10/98	9/16/98	9/23/98	10/1/98
No estrogen, no IGF-1	1	2	3	4	5	6	7	8	9
NOD1	0	0	0	0	0	palp	3X3	3X3	5X5
NOD2	0	0	0	0	0	palp	3X3	4X4	6X5
NOD3	0	0	0	palp	0	2X2	4X4	5X5	7X7
NOD4	0	0	0	palp	2X2	3X3	6X6	7X7	9X7
NOD5	0	0	0	2X2	2X2	6X6	6X6	7X7	8X8
No estrogen, plus IGF-1									
NOD6	palp	palp	palp	5X5	5X6	8X8	9X9	8X8	10X10
NOD7	palp	palp	5X3	5X5	7X4	9X7	9X9	9X9	13 X10
NOD8	palp	palp	4X4	5X5	5X6	8X8	9X8	7X7	9X13
NOD9	palp	3X4	6X4	6X5	7X4	8X6	8X11	11X7	13X10
NOD10	0	2X2	5X5	5X5	4X4	8X8	10X8	11X7	10X6
Plus estrogen, plus IGF-1									
NOD11	0	palp	0	palp	3X3	6X6	8X7	9X10	11X10
NOD12	palp	palp	palp	5X5	7X5	6X7	10X10	8X8	12X11
NOD13	palp	palp	3X4	5X5	6X5	8X7	10X10	9X9	10X10
NOD14	palp	4X4	5X5	5X4	5X5	9X8	10X10	9X9	10X10
NOD15	palp	4X4	4X4	6X5	7X5	8X8	11X6	dead	dead

Animal									
No estrogen, no IGF-1									
NOD1	0	0	0	0	0	0.78	21	21	98
NOD2	0	0	0	0	0	0.78	21	50	141
NOD3	0	0	0	0.78	0	6.3	50	98	269
NOD4	0	0	0	0.78	6.3	21.2	169	269	445
NOD5	0	0	0	6.3	6.3	169	169	269	401
Average tumor Volume	0	0	0	1.572	2.52	39.612	86	141.4	270.8
Standard deviation				1.8912	3.024	51.7552	66.4	102.08	121.76
No estrogen, plus IGF-1									
NOD6	0.78	0.78	0.78	98	117	401	572	401	785
NOD7	0.78	0.78	58.8	98	153	445	572	572	1326
NOD8	0.78	0.78	50.2	98	117	401	508	269	826
NOD9	0.78	28.3	113	141	153	301	552	664	1326
NOD10	0	6.3	98	98	153	401	628	664	471
Average tumor Volume	0.624	7.388	64.156	106.6	138.6	389.8	566.4	514	946.8
Standard deviation	0.348827	11.93202	44.077	13.76	17.28	35.52	29.12	143.2	303.36
Plus estrogen, plus IGF-1									
NOD11	0	0.78	0	0.78	21.2	169	351	635	949
NOD12	0.78	0.78	0.78	98	192	197	785	402	1243
NOD13	0.78	0.78	28.2	98	141	351	785	572	785
NOD14	0.78	50.2	98.1	78.5	98	508	785	572	785
NOD15	0.78	50.2	50.2	141	192	401	569	dead	
Average tumor volume	0.624	20.548	35.456	83.256	128.84	325.2	655	545.25	940.5
Standard deviation	0.348827	27.06845	40.78559	34.8928	55.392	113.76	156	71.625	155.5

FIGURE IX

The effect of continuous infusion human IGF1 on MCF7R engraftment and growth in NOD scid/scid mice

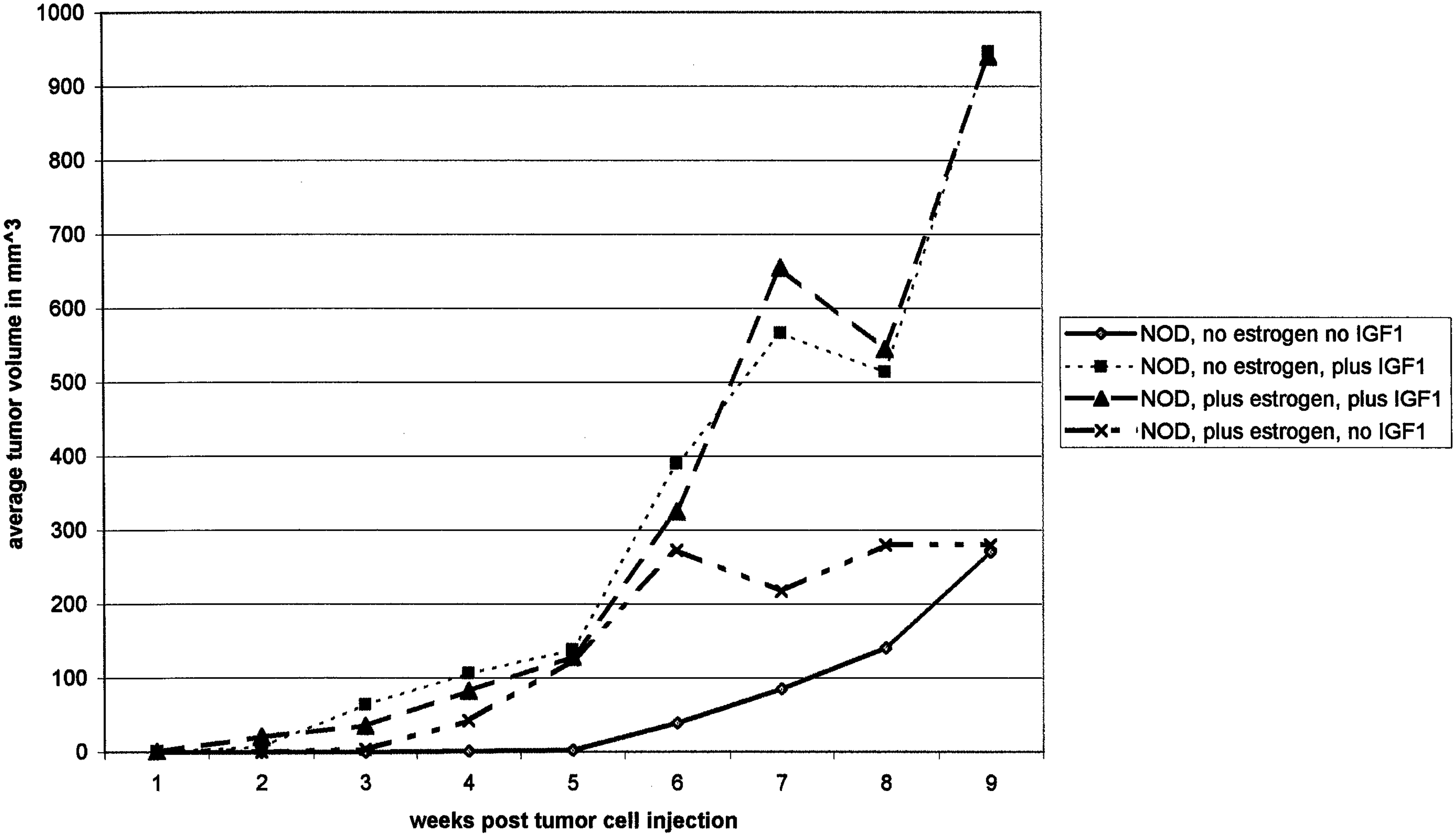


TABLE X: Tumor measurements in *scid/scid lit/lit* mice exposed to continuous infusion IGF1 and/or 17 beta estradiol

Tumor cells 2 X 10^5 MCF-7R cells injected into the mammary fat pad on 7/29/98

Weeks post injection of tumor cells

Animal	8/6/98	8/13/98	8/19/98	8/25/98	9/1/98	9/8/98	9/16/98	9/23/98	10/1/98	
No estrogen, no IGF-1	1	2	3	4	5	6	7	8	9	10
lit/lit1	0	palp	palp	palp	3X3	6X6	6X6	7X8	7X5	
lit/lit2	0	palp	palp	2X2	3X3	4X4	6X6	6X7	6X6	
lit/lit3	0	0	0	3X3	3X4	4X4	4X4	5X6	5X5	
lit/lit4	0	0	0	palp	palp	3X3	4X4	6X6	5X7	
lit/lit5	0	0	0	palp	palp	3X3	4X4	6X6	5X4	
No estrogen, plus IGF-1										
lit/lit6	palp	palp	palp	3X3	3X3	6X6	6X8	9X6	11X6	
lit/lit7	palp	palp	0	2X2	2X2	2X7	7X7	8X8	8X10	
lit/lit8	palp	palp	2X2	4X4	3X3	6X6	8X7	10X11	10X10	
lit/lit9	palp	3X3	5X5	5X5	5X5	8X6	10X7	10X10	8X11	
lit/lit10	palp	1X1	4X4	6X5	7X4	8X8	9X9	dead		
Plus estrogen, plus IGF-1										
lit/lit11	5X4	3X3	4X5	8X6	7X6	7X7	8X8	dead	dead	
lit/lit12	palp	3X4	4X3	5X5	7X5	8X6	8X8	8X8	8X11	
lit/lit13	palp	3X3	3X3	5X4	6X6	5X8	8X7	9X9	12X8	
lit/lit14	palp	palp	2X2	5X5	5X5	8X7	10X7	10X10	9X12	
lit/lit15	palp	palp	2x2	4X4	4X5	7X10	8X12	7X10	10X8	

Animal	8/6/98	8/13/98	8/19/98	8/25/98	9/1/98	9/8/98	9/16/98	9/23/98	10/1/98	
No estrogen, no IGF-1	1	2	3	4	5	6	7	8	9	10
lit/lit1	0	0.78	0.78	0.78	21	169	169	230	192	
lit/lit2	0	0.78	0.78	6.3	21	50	169	197	169	
lit/lit3	0	0	0	21	28	50	50	117	98	
lit/lit4	0	0	0	0.78	0.78	21	50	169	137	
lit/lit5	0	0	0	0.78	0.78	21	50	169	78.5	
Average tumor volume	0	0.312	0.312	5.928	14.312	52.83333	97.6	176.4	134.9	
Standard deviation		0.427224	0.427224	8.757986	12.67922	38.72222	57.12	29.68	37.32	
No estrogen, plus IGF-1										
lit/lit6	0.78	0.78	0.78	21	21	169	226	381	569	
lit/lit7	0.78	0.78	0	6.3	5.3	22	269	402	502	
lit/lit8	0.78	0.78	6.3	50.2	21	169	351	863	785	
lit/lit9	0.78	21.2	98.1	98	98	301	549	785	552	
lit/lit10	0.78	0.78	62.8	141	153	401	572	dead	dead	
Average tumor volume	0.78	4.864	33.596	63.3	59.66	212.4	393.4	607.75	602	
standard deviation	0	9.132102	44.62144	55.79265	63.4951	110.88	133.68	216.25	91.5	
Plus estrogen, plus IGF-1										
lit/lit11	78.5	21.2	62.8	301	230	269	402	dead	dead	
lit/lit12	0.78	28.2	37.6	98	192	301	402	402	552	
lit/lit13	0.78	21.2	21.2	78	169	157	351	572	904	
lit/lit14	0.78	0.78	6.3	98	98	351	549	785	736	
lit/lit15	0.78	0.78	6.3	50	62.8	384	602	384	628	
Average tumor volume	16.324	14.432	26.84	125	150.36	292.4	461.2	535.75	705	
Standard deviation	34.75744	12.78597	23.89818	70.4	55.968	63.52	91.44	142.75	115	

FIGURE X

The effect of continuous infusion human IGF1 on MCF7R engraftment and growth in scid/scid lit/lit mice

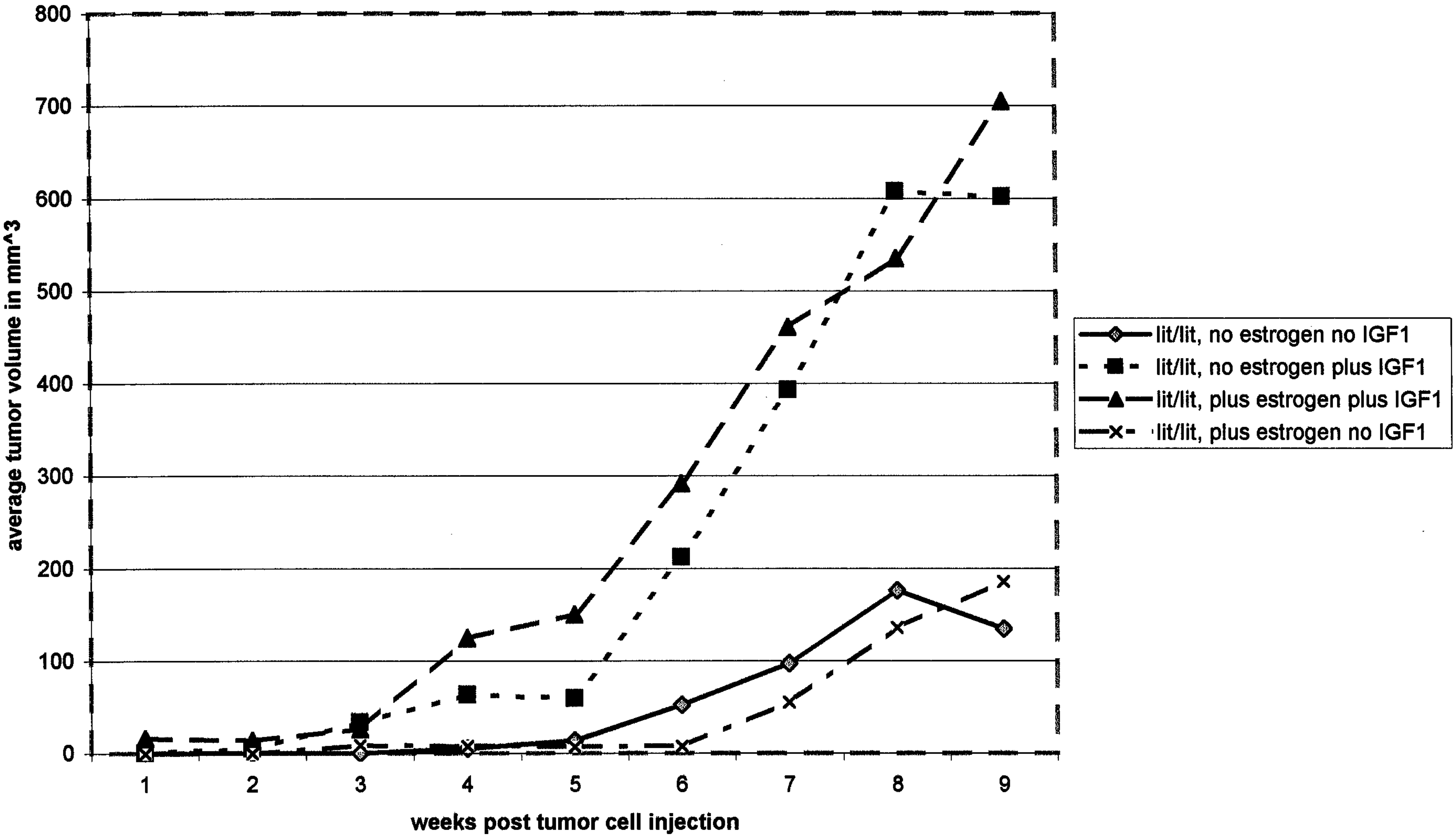


TABLE XI: Tumor measurements in *scid/scid lit +/-* mice exposed to continuous infusion IGF1 and /or 17 beta estradiol

Tumor cells 2 X 10^5 MCF-7R cells injected into the mammary fat pad on 7/29/98

	Weeks post injection of tumor cells								
Animal	8/6/98	8/13/98	8/19/98	8/25/98	9/1/98	9/10/98	9/16/98	9/23/98	
No estrogen, no IGF-1	1	2	3	4	5	6	7	8	9	10
LIT+/-1	0	palp	palp	3X3	2X2	5X5	6X6	8X7	11X6	
LIT +/-2	0	palp	2X2	5X4	3X3	4X4	6X6	8X7	6X6	
LIT +/-3	0	0	0	palp	palp	5X4	6X6	7X7	7X6	
LIT+/- 4	0	0	0	palp	palp	4X4	5X5	7X5	8X9	
LIT+/- 5	0	0	0	palp	0	3X2	4X4	5X5	4X6	
No estrogen, plus IGF-1										
LIT+/-6	palp	palp	2X2	5X5	5X7	7X7	8X8	9X8	10X9	
LIT+/- 7	palp	palp	0	palp	2X2	4X2	3X3	4X4	5X5	
LIT+/-8	palp	palp	palp	3X3	3X3	5X4	7X7	7X7	10X7	
LIT+/- 9	palp	0	0	3X3	5X5	8X8	7X7	11X8	12X8	
LIT+/- 10	4X4	2X2	2X2	4X4	5X5	6X6	11X8	12X7	7X9	
Plus estrogen, plus IGF-1										
LIT+/-11	0	2X3	4X4	7X5	7X5	7X8	9X9	dead	dead	
LIT+/-12	palp	4X4	5X3	5X4	7X5	8X5	10X6	12X9	13X11	
LIT+/-13	palp	4X3	4X4	3X3	7X5	10X8	11X10	11X7	12X10	
LIT+/-14	0	palp	4X3	6X4	7X6	9X8	10X7	11X7	10X9	
LIT+/-15	0	5X3	5X3	5X5	7X6	9X6	7X9	10X10	10X10	
Animal										
No estrogen, no IGF-1										
LIT+/-1	0	0.78	0.78	21.2	6.3	98	169	351	569	
LIT +/-2	0	0.78	6.3	78.5	21.2	50	169	351	169	
LIT +/-3	0	0	0	0.78	0.78	78	169	269	230	
LIT+/- 4	0	0	0	0.78	0.78	50	98	192	452	
LIT+/- 5	0	0	0	0.78	0	14	50	98	75	
Average tumor volume	0	0.312	1.416	20.408	5.812	58	131	252.2	299	
Standard deviation		0.427224	2.751051	23.5536	6.3504	24	45.6	85.76	169.2	
No estrogen, plus IGF-1										
LIT+/-6	0.78	0.78	6.3	98	137	269	402	508	706	
LIT+/- 7	0.78	0.78	0	0.78	6.3	25	21	50	98	
LIT+/-8	0.78	0.78	0.78	21	21	78	269	269	549	
LIT+/- 9	0.78	0	0	211	98	401	269	759	904	
LIT+/- 10	50.2	6.3	6.3	50.2	98	169	759	791	346	
Average tumor volume	10.664	1.8288	2.8992	76.196	72.06	188.4	344	475.4	520.6	
Standard deviation	22.1013	2.578046	3.323534	62.6432	46.728	117.28	189.2	252.72	238.88	
Plus estrogen, plus IGF-1										
LIT+/-11	0	9.4	50.2	192	192	307	572	dead	dead	
LIT+/-12	0.78	50.2	58.9	78.5	192	251	471	1017	1459	
LIT+/-13	0.78	37.9	50.2	21	192	628	949	664	1130	
LIT+/-14	0	0.78	37.9	113	230	508	549	664	706	
LIT+/-15	0	58.9	58.9	98	230	381	346	785	785	
Avrage tumor volume	0.312	31.436	51.22	100.5	207.2	415	577.4	782.5	1020	
Standard deviation	0.427224	25.36493	8.62363	41.6	18.24	122.4	148.64	118.5	345.7562	
							225.7151			

FIGURE XI

The effect of continuous infusion human IGF1 on MCF7R engraftment and growth in scid/scid lit +/- mice

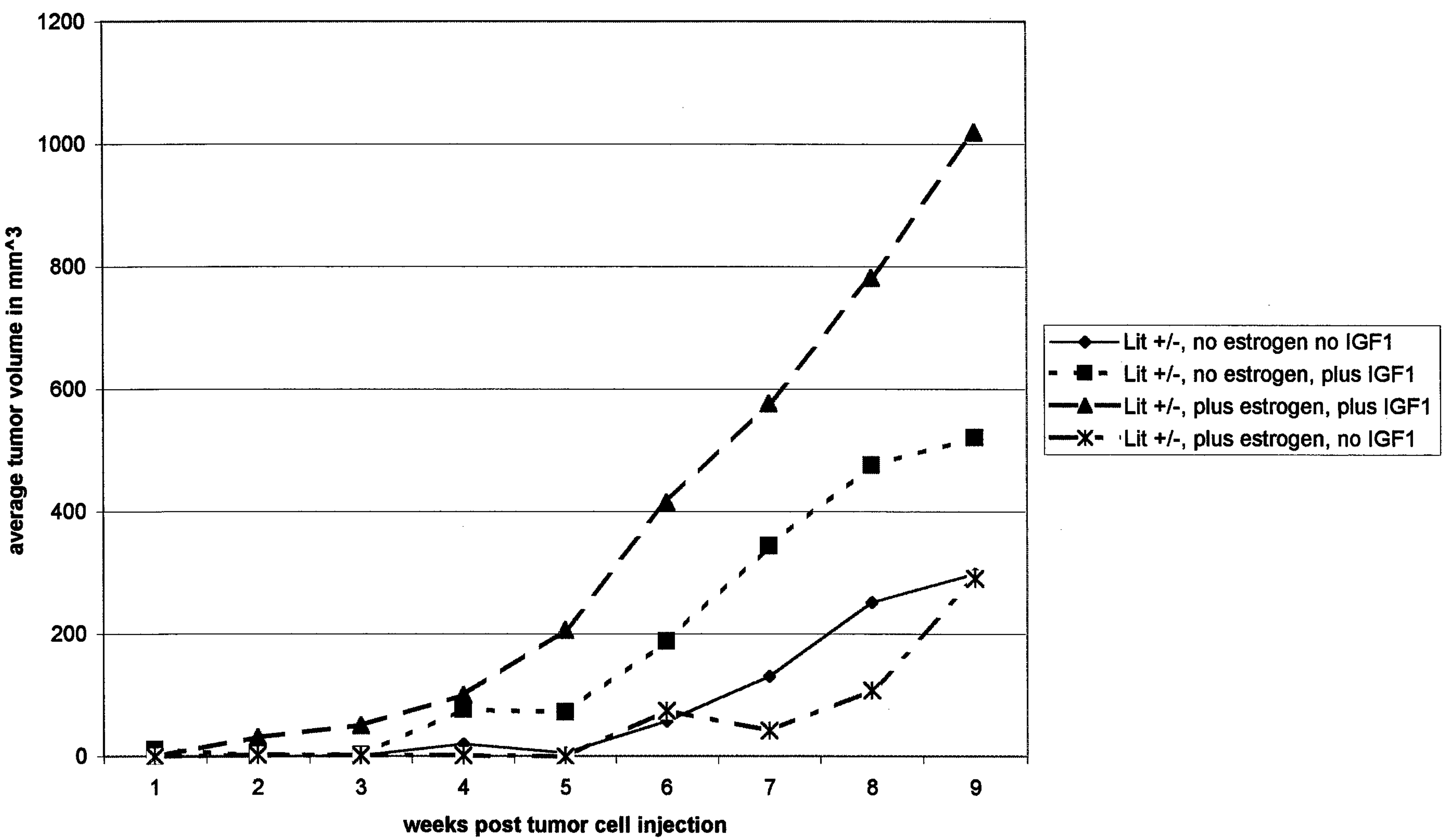


FIGURE XII

Nested RT-PCR Assay for IGFR From Tumor Samples Obtained From Animals Treated With Bolus rhGH

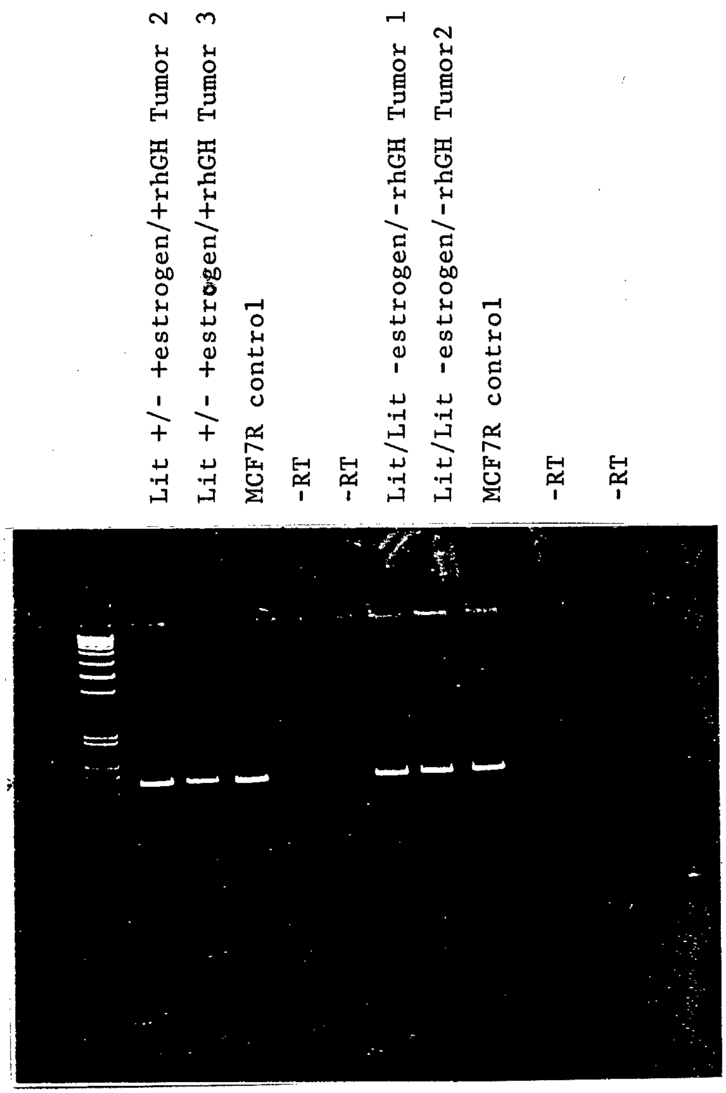


FIGURE XIII

Nested RT-PCR Assay for IGF 2 From Tumor Samples Obtained From Animals Treated With Bolus rhGH

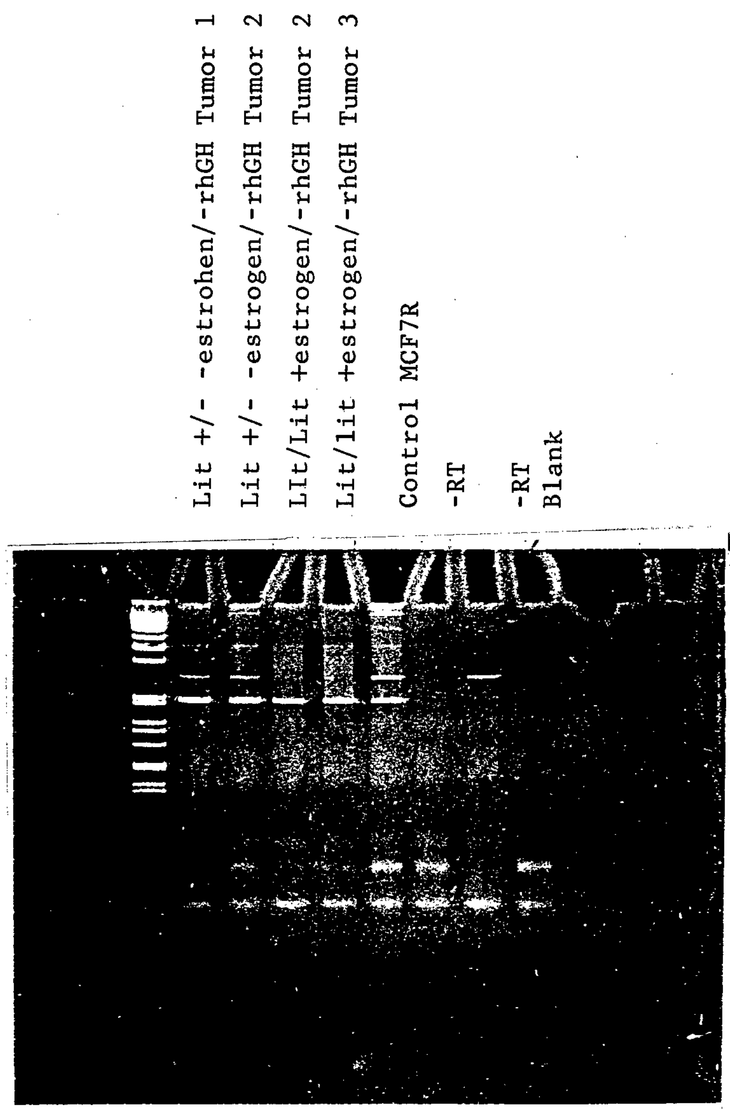
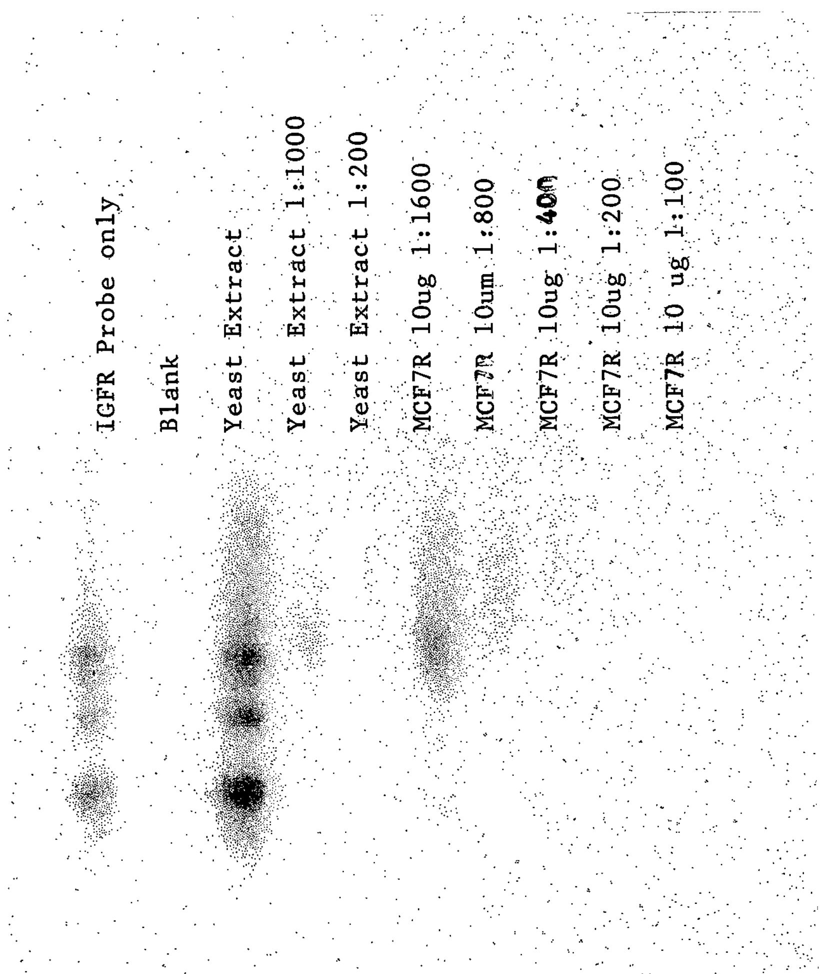


FIGURE XIV

Initial Attempt To Develop RNA Protection Assay For IGFR



APPENDIX

STATEMENT OF WORK

Technical Objectives (Specific Aims) 1-3

Task 1: Months 1-4:	Implant MCF-7R tumor cells into experimental animals Initiate experiments in Aim 1 with rhGH given by bolus or continuous infusion. Measure serum levels of GH.
Task 2: Months 1-4:	Synthesize probes for detection of hGH and IGF-1 for use in northern and western analyses. Test probes for efficacy on positive and negative control specimens.
Task 3: Months 5-8:	Implant MCF-7R tumor cells into experimental animals. Initiate experiments in Aim 2 with IGF-1 Measure serum levels of IGF-1
Task 4: Months 5-8:	Determine if additional dose levels of rhGH could optimize results. If so, set-up experimental animals to repeat experiments in Aim 1 at higher or lower dose of rhGH.
Task 5: Months 5-8:	Perform northern and western analyses on tumors from animals in Specific Aim 1. Probe with GH probe.
Task 6: Months 9-12:	Implant MCF-7R tumor cells into experimental animals. Initiate experiments in Aim 3 with IGF-1/17-β estradiol.
Task 7: Months 9-12:	Determine if additional dose levels of IGF-1 could optimize experimental results. If so, set-up experimental animals to repeat experiments in Aim 1 at higher or lower dose of IGF-1.
Task 8: Months 9-12:	Perform northern and western analyses on tumors from animals in Specific Aim 2. with IGF-1 probe.
Task 9: Months 13-16:	Perform northern and western analyses on tumors from animals in Specific Aim 3. Probe with IGF-1 probe.

Task 10: Months 13-16:	Repeat any experiments in Aims 1-3 that could help to further optimize the experimental model
Task 11: Months 13-20	Perform northern and western analyses on animals studied in Task 4,7.
Task 12: Months 15-24:	Implement optimized experimental parameters in animal model. Begin implanting primary breast cancers into optimized animal model.